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(54) Title: NOVEL HUMAN VOLTAGE-GATED POTASSIUM CHANNEL (57) Abstract The present invention is directed to novel human DNA sequences encoding a voltage-gated potassium channel, KCNQ5, located in a chromosomal region that contains a gene associated with Stargardt-like macular dystrophy, cone-rod macular dystrophy, and Salla disease.		

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TITLE OF THE INVENTION
NOVEL HUMAN VOLTAGE-GATED POTASSIUM CHANNEL

CROSS-REFERENCE TO RELATED APPLICATIONS

5 Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

10 REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

15 The present invention is directed to novel human DNA sequences encoding a voltage-gated potassium channel.

BACKGROUND OF THE INVENTION

20 Voltage-gated potassium channels form transmembrane pores that open in response to changes in cell membrane potential and selectively allow potassium ions to pass through the membrane. Many voltage-gated potassium channels have been identified. They are distinguishable by tissue-specific patterns of expression as well as by electrophysiological and pharmacological properties.

25 Voltage-gated potassium channels have been shown to be involved in maintaining cell membrane potentials and controlling the repolarization of action potentials in many cells, *e.g.*, neurons, muscle cells, and pancreatic β cells. They are important targets for drug discovery in connection with a variety of diseases.

30 Functional voltage-gated potassium channels are believed to be tetramers of four alpha subunits, each of which contains six transmembrane spanning segments. The alpha subunits making up a tetramer may be the same (in the case of homotetramers) or may be different (in the case of heterotetramers). The membrane-spanning alpha subunits making up the tetramers may sometimes be associated with additional, beta subunits, which may alter the behavior of the tetramers.

For reviews of voltage-gated potassium channels see Robertson, 1997, Trends Pharmacol. Sci. 18:474-483; Jan & Jan, 1997, J. Physiol. 505:267-282; Catterall, 1995, Ann. Rev. Biochem. 64:493-531.

Macular dystrophy is a term applied to a heterogeneous group of diseases that collectively are the cause of severe visual loss in a large number of people. A common characteristic of macular dystrophy is a progressive loss of central vision resulting from the degeneration of the pigmented epithelium underlying the retinal macula. In many forms of macular dystrophy, the end stage of the disease results in legal blindness. More than 20 types of macular dystrophy are known: e.g., age-related macular dystrophy, Stargardt's and Stargardt-like macular dystrophy, cone-rod dystrophies, atypical vitelliform macular dystrophy (VMD1), Usher Syndrome Type 1B, autosomal dominant neovascular inflammatory vitreoretinopathy, familial exudative vitreoretinopathy, and Best's macular dystrophy. For a review of the macular dystrophies, see Sullivan & Daiger, 1996, Mol. Med. Today 2:380-386.

Cone-rod dystrophies involve an initial loss of cone photoreceptors followed by the degeneration of rod photoreceptors. This loss of photoreceptors can lead to blindness. Cone-rod dystrophies appear to be a heterogeneous group of inherited disorders for which multiple chromosomal locations have been implicated (Evans et al., 1994, Nature Genet. 6:210-213; Kelsell et al., 1997, Hum. Mol. Genet. 6:597-600). In particular, Kelsell et al., 1998, Am. J. Hum. Genet. 63:274-279 found a candidate gene (CORD7) located at chromosome 6q in a four-generation British family affected with cone-rod dystrophy. A marker in 6q, D6S280, showed a high LOD score of 3.31 (at genetic distance = 0).

Stargardt-like macular dystrophy is an inherited, dominant retinal disease. Affected individuals have normal vision in early childhood but show impaired central vision either in late childhood or early adulthood. The first observable characteristics of the disease are flecks seen in the macula. This is followed by central atrophy, resulting in visual acuity decreasing to 20/200 or worse (Stone et al., Arch. Ophthalmol. 112:765-772 [Stone]). Stone mapped a gene responsible for Stargardt-like macular dystrophy to chromosome 6q. The marker D6S280 was observed by Stone to have the high LOD score of 5.5 (at genetic distance = 0).

Cone-rod dystrophy and Stargardt-like macular dystrophy appear different from a clinical perspective. For example, Stargardt-like macular dystrophy

generally begins in childhood and involves white/yellow flecks in the retina while cone-rod dystrophy is an adult-onset disorder in which no flecks are present. Despite such clinical differences, both diseases may be caused by mutations in the same gene. It is not uncommon for different mutations in a single gene to give rise to clinically different disorders. For example, depending upon the particular mutation, mutations in the peripherin/RDS gene can give rise to either butterfly-shaped pigment dystrophy of the fovea, retinitis pigmentosa, pattern dystrophy, flavus maculatus, macular dystrophy, or central areolar choroidal dystrophy (Nichols et al., 1993, *Nature Genet.* 3:202-207; Weleber et al., 1993, *Arch. Ophthalmol.* 111:1531-1542; Wells et al., 1993, *Nature Genet.* 3:213-218; Reig et al., 1995, *Ophthalmic. Genet.* 16:39-44).

While studies of macular dystrophies such as cone-rod dystrophy or Stargardt-like macular dystrophy are valuable in themselves, such studies are also valuable in that they are expected to shed light on age-related macular degeneration (AMD). AMD is the leading cause of severe visual loss in older individuals. Genetic factors apparently play a role in AMD (Hyman et al., 1983, *Am. J. Epidemiol.* 118:213-227; Gass, 1973, *Arch. Ophthalmol.* 90:206-217). It is believed likely that mild allelic variations of such earlier-onset diseases as cone-rod dystrophy and Stargardt-like macular dystrophy are responsible for some cases of AMD. Thus, understanding and developing treatments for these earlier-onset diseases should prove valuable with respect to AMD as well.

Salla disease is a recessive condition characterized by early-onset psychomotor retardation and ataxia that involves defects in the lysosomal transport of sialic acid. Leppänen et al., 1996, *Genomics* 37:62-67 (Leppänen) located the gene for Salla disease in the immediate vicinity of the marker D6S280. Leppänen screened a PAC library with the marker D6S280 and obtained three positive clones, among which were PAC 141B1 and PAC 224H23, strongly suggesting that the gene for Salla disease is present on these PACs.

SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences encoding a voltage-gated potassium channel, KCNQ5, located in a chromosomal region that contains a gene associated with Stargardt-like macular dystrophy, cone-rod macular dystrophy, and Salla disease.

The present invention includes genomic KCNQ5 DNA as well as cDNA that encodes the KCNQ5 protein. The human genomic KCNQ5 DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The human cDNA encoding KCNQ5 protein is substantially free
5 from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2. Also provided is KCNQ5 protein encoded by the novel DNA sequences. The human KCNQ5 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3. Methods of expressing KCNQ5 protein in recombinant systems are provided as well as methods of identifying activators and
10 inhibitors of KCNQ5 protein function. Also provided are diagnostic methods that detect carriers of mutant KCNQ5 genes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-AO shows the genomic DNA sequence of human KCNQ5
15 (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon in exon 1 and the stop TAA codon in exon 14 are shown in bold italics. The D6D280 genetic marker and a phosphoglycerate pseudogene are underlined in bold. The exact lengths of the gaps between exons 1 and 2, 2 and 3, 10 and 11, 11 and 12, 12 and 13, and 13 and 14 are unknown. These gaps are represented as runs of ten
20 bold ns for the sake of convenience.

Figure 2A-E shows the nucleotide sequence (SEQ.ID.NO.:2) and encoded amino acid sequence (SEQ.ID.NO.:3) of human KCNQ5 cDNA. The ATG start codon is at position 138; the TAA stop codon is at position 2,676.

Figure 3A shows the results of a Northern blot of KCNQ5 mRNA
25 expression in various human tissues. Figure 3B shows the results of RT-PCR analysis of KCNQ5 mRNA expression in various human tissues.

Figure 4A shows a sequence alignment of human KCNQ5 protein
(SEQ.ID.NO.:3) with human KCNQ4 protein (SEQ.ID.NO.:4). The consensus
sequence shown is (SEQ.ID.NO.:5). Figure 4B-C shows a multiple sequence
30 alignment between human KCNQ5 protein (SEQ.ID.NO.:3), human KCNQ1 protein (SEQ.ID.NO.:43), human KCNQ2 protein (SEQ.ID.NO.:6), human KCNQ3 protein (SEQ.ID.NO.:7), and human KCNQ4 protein (SEQ.ID.NO.:4). The consensus sequence shown is (SEQ.ID.NO.:8).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

“Substantially free from other proteins” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins.

- 5 Thus, a KCNQ5 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- KCNQ5 proteins. Whether a given KCNQ5 protein preparation is
- 10 substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

- “Substantially free from other nucleic acids” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other
- 15 nucleic acids. Thus, a KCNQ5 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non- KCNQ5 nucleic acids. Whether a given KCNQ5 DNA preparation is substantially free from other nucleic acids can be
- 20 determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

- A “conservative amino acid substitution” refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples
- 25 of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

- 30 A polypeptide has “substantially the same biological activity as KCNQ5” if that polypeptide conducts a voltage-gated potassium current when expressed in appropriate cell types and has an amino acid sequence that is at least about 50% identical to SEQ.ID.NO.:3 when measured by such standard programs as BLAST or FASTA.

The present invention relates to the identification and cloning of KCNQ5, a gene encoding a novel voltage-gated potassium channel. The human KCNQ5 gene is located on chromosome 6q14, in a chromosomal region that contains genes that have been linked with the occurrence of at least three diseases: Stargardt-like macular dystrophy, cone-rod dystrophy, and Salla disease.

The human KCNQ5 gene is present on PAC clones from chromosomal region 6q14. PAC141B1 was sequenced and KCNQ5 was found based on homology between the genomic sequences of KCNQ5 present in PAC 141B1 and the sequences of known potassium channel genes. PAC 141B1 is available from Research Genetics, Inc., Huntsville, AL, as an individual clone from the RPCI4,5,6 Library (catalog number CTLI.C). Using PCR primers derived from the KCNQ5 sequence, a cDNA sequence representing the coding region as well as a large portion of the 3'-UTR of KCNQ5 was isolated from a human fetal brain cDNA library. Comparison of this cDNA clone with the genomic sequences present in PAC141B1, as well as KCNQ5 sequences found in PAC224H23, showed that exons 3-11 and portions of flanking intronic regions are present in PAC141B1. Exon 2 and flanking intronic regions were found in PAC224H23, while the rest of the KCNQ5 gene (exons 1, 12-14, and flanking intronic regions) was recovered from total human genomic DNA by using cDNA primers and a GenomeWalker kit from Clontech, Palo Alto, CA.

PAC141B1 and PAC224H23 are located in the region of the Salla disease gene (Leppänen et al., 1996, Genomics 37:62-67). PAC141B1 contains the polymorphic genetic marker D6S280 that is located in intron 3 of the KCNQ5 gene between exons 3 and 4 (Figure 1). D6S280 is the marker that detects the maximum LOD score of 5.5 (at genetic distance = 0) in families with Stargardt-like macular dystrophy (Stone et al., Arch. Ophthalmol. 112:765-772). D6S280 also detects a LOD score of 3.31 (at genetic distance = 0) in families with cone-rod dystrophy (Kelsell et al., 1998, Am. J. Hum. Genet. 63:274-279). These LOD scores indicate that D6S280 is very closely linked to, and probably is within, the gene for Stargardt-like macular dystrophy and cone-rod dystrophy. In view of these findings, it is likely that KCNQ5 is involved in Salla disease, Stargardt-like macular dystrophy, and cone-rod dystrophy.

That KCNQ5 should be involved with these three diseases is consistent with its expression pattern (see Figure 3A-B) which shows that KCNQ5 is expressed predominately in the retina and brain, in addition to being expressed in the

skeletal muscle. Stargardt-like macular dystrophy and cone-rod dystrophy are inherited retinal diseases while Salla disease is a disorder that is characterized by early onset psychomotor retardation and ataxia.

Bioinformatic analysis revealed a striking homology of the KCNQ5 protein to a group of voltage gated potassium channels (KCNQ1, KCNQ2, KCNQ3, and KCNQ4; see Figure 4A-B). All of the typical amino acid motifs of these potassium channels are preserved in KCNQ5. A Kyte-Doolittle algorithm analysis predicts a transmembrane organization for KCNQ5 that is typical for this group of potassium channels. Mutations in members of this family of potassium channels have been shown to result in inherited disease (KCNQ2 and KCNQ3, epilepsy [Biervert et al., 1998, Science 279:403-406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690]; KCNQ4, a form of nonsyndromic dominant deafness [Kubisch et al., 1999, Cell 96:437-446], KCNQ1, congenital long QT syndrome which causes cardiac arrhythmias and sudden death [Splawski et al., 1997, N. Engl. J. Med. 336:1562-1567]).

The present invention provides DNA encoding KCNQ5 that is substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding KCNQ5. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 14 exons. These exons collectively have an open reading frame that encodes a protein of 846 amino acids.

The present invention includes cDNA encoding KCNQ5 protein. Such a cDNA is shown in Figure 2 as SEQ.ID.NO.:2. The present invention therefore includes DNA comprising the nucleotide sequence SEQ.ID.NO.:2. The DNA can be isolated or substantially free of other DNA sequences.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding region of SEQ.ID.NO.:2. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 138-2,675 of SEQ.ID.NO.:2. Also included are recombinant DNA molecules having a nucleotide sequence comprising positions 138-2,675 of SEQ.ID.NO.:2 and isolated DNA molecules having a nucleotide sequence comprising positions 138-2,675 of SEQ.ID.NO.:2.

The novel DNA sequences of the present invention encoding KCNQ5, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which KCNQ5 is not naturally linked, to form "recombinant DNA molecules" encoding KCNQ5. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NO:1 or SEQ.ID.NO:2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the KCNQ5 protein where the nucleotide

sequence of the synthetic DNA differs significantly from the nucleotide sequences of SEQ.ID.NO:2, but still encodes the same KCNQ5 protein as SEQ.ID.NO:2. Such synthetic DNAs are intended to be within the scope of the present invention.

5 Mutated forms of SEQ.ID.NO:1 or SEQ.ID.NO:2 are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NO:1 or SEQ.ID.NO:2 which give rise to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration are within the scope of the present invention.

10 Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding KCNQ5 protein. Such recombinant host cells can be cultured under suitable conditions to produce KCNQ5 protein. An expression vector containing DNA encoding KCNQ5 protein can be used for expression of KCNQ5 protein in a recombinant host cell.

15 Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, amphibian cells such as *Xenopus* oocytes, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cells and cell lines which are suitable for recombinant expression of KCNQ5 protein and which are widely mavailable,

20 include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171),

25 ARPE-19 human retinal pigment epithelium (ATCC CRL-2302), *Xenopus* melanophores, and *Xenopus* oocytes.

A variety of mammalian expression vectors can be used to express recombinant KCNQ5 in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo

30 (Stratagene), pSG5 (Stratagene), pcDNA1 and pcDNA1amp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Another suitable vector is the PT7TS oocyte expression vector. Following expression in recombinant cells, KCNQ5 can

be purified by conventional techniques to a level that is substantially free from other proteins.

Certain voltage-gated potassium channel subunits have been found to require the expression of other voltage-gated potassium channel subunits as "chaperones" in order to be properly expressed at high levels and inserted in membranes. For example, co-expression of KCNQ3 appears to enhance the expression of KCNQ2 in *Xenopus* oocytes (Wang et al., 1998, Science 282:1890-1893). Also, some voltage-gated potassium channel Kv1 α subunits require other related alpha subunits or Kv β 2 subunits (Shi et al., 1995, Neuron 16:843-852). Accordingly, the recombinant expression of the KCNQ5 protein may under certain circumstances benefit from the co-expression of other voltage-gated potassium channel proteins and such co-expression is intended to be within the scope of the present invention.

The present invention includes KCNQ5 protein substantially free from other proteins. The amino acid sequence of the full-length KCNQ5 protein is shown in Figure 2 as SEQ.ID.NO.:3. Thus, the present invention includes KCNQ5 protein substantially free from other proteins having the amino acid sequence SEQ.ID.NO.:3. The present invention also includes isolated KCNQ5 protein having the amino acid sequence SEQ.ID.NO.:3.

Mutated forms of KCNQ5 proteins are intended to be within the scope of the present invention. In particular, mutated forms of SEQ.ID.NO.:3 that give rise to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration are within the scope of the present invention.

As with many proteins, it is possible to modify many of the amino acids of KCNQ5 and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified KCNQ5 proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as KCNQ5. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as

KCNQ5. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO:3 wherein the polypeptides still retain substantially the same biological activity as KCNQ5. In particular, the present invention includes embodiments where the above-described substitutions are

5 conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in KCNQ5 is also present in the corresponding position of any one of KCNQ1, KCNQ2, KCNQ3, or KCNQ4 (see Figure 4A-B).

The KCNQ5 proteins of the present invention may contain post-

10 translational modifications, *e.g.*, covalently linked carbohydrate, phosphorylation, myristoylation, *etc.*.

The present invention also includes chimeric KCNQ5 proteins. Chimeric KCNQ5 proteins consist of a contiguous polypeptide sequence of at least a portion of KCNQ5 protein fused to a polypeptide sequence of a non-KCNQ5 protein.

15 The present invention also includes isolated forms of KCNQ5 proteins and KCNQ5 DNA. Use of the term "isolated" indicates that KCNQ5 protein or KCNQ5 DNA has been removed from its normal cellular environment. Thus, an isolated KCNQ5 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply

20 that an isolated KCNQ5 protein is the only protein present. but instead means that an isolated KCNQ5 protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the KCNQ5 protein. Thus, a KCNQ5 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is

25 an "isolated KCNQ5 protein."

It is known that other members of the family of potassium channels to which KCNQ5 belongs can interact to form heteromeric structures resulting in functional potassium channels. For example, KCNQ2 and KCNQ3 can assemble to form a heteromeric functional potassium channel (Wang et al., 1998, Science

30 282:1890-1893). Accordingly, it is believed likely that KCNQ5 will also be able to form heteromeric structures with other proteins where such heteromeric structures constitute functional potassium channels. Thus, the present invention includes such heteromers comprising KCNQ5. Preferred heteromers are those in which KCNQ5 forms heteromers with at least one of KCNQ1, KCNQ2, KCNQ3, or KCNQ4.

A cDNA fragment encoding full-length KCNQ5 can be isolated from a human retinal or brain cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for KCNQ5 shown in Figure 2 as SEQ.ID.NO.:2. Suitable primer pairs would be, *e.g.*:

5'-GGGGGCCCCGGATGAGCC-3' (SEQ.ID.NO.:9) and
5'-GAAGAACTTATTTTCAGTTTGA-3' (SEQ.ID.NO.:10)

The above primers are meant to be illustrative only; one skilled in the art would readily be able to design other suitable primers based upon SEQ.ID.NO.:2. Such primers could be produced by methods of oligonucleotide synthesis that are well known in the art.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl₂, 200 μ M for each dNTP, 50 mM KCl, 0.2 μ M for each primer, 10 ng of DNA template, 0.05 units/ μ l of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding KCNQ5 can be isolated would be Human Retina 5'-stretch cDNA library in lambda gt10 or lambda gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA) or human fetal brain 5-stretch plus cDNA library (catalog number HL5024t, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of 846 amino acids (SEQ.ID.NO.:3) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). KCNQ5 protein can then be produced by transferring an expression

vector encoding KCNQ5 or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. KCNQ5 protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone
5 encoding KCNQ5 can be isolated from a cDNA library using as a probe
oligonucleotides specific for KCNQ5 and methods well known in the art for screening
cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*,
Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring
Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA*
10 *Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II.
Oligonucleotides that are specific for KCNQ5 and that can be used to screen cDNA
libraries can be readily designed based upon the cDNA sequence of KCNQ5 shown in
Figure 2 as SEQ.ID.NO.:2 and can be synthesized by methods well-known in the art.

Genomic clones containing the KCNQ5 gene can be obtained from
15 commercially available human PAC or BAC libraries available from Research
Genetics, Huntsville, AL. PAC clones containing the KCNQ5 gene (*e.g.*, PAC141B1,
PAC224H23) are commercially available from Research Genetics, Huntsville, AL
(catalog number for individual PAC clones is RPCI.C). Alternatively, one may
prepare genomic libraries, especially in P1 artificial chromosome vectors, from which
20 genomic clones containing the KCNQ5 can be isolated, using probes based upon the
KCNQ5 sequences disclosed herein. Methods of preparing such libraries are known
in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).

The novel DNA sequences of the present invention can be used in
various diagnostic methods relating to Stargardt-like macular dystrophy, cone-rod
25 dystrophy, Salla disease, or age-related macular degeneration. The present invention
provides diagnostic methods for determining whether a patient carries a mutation in
the KCNQ5 gene that predisposes that patient toward the development of Stargardt-
like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular
degeneration. In broad terms, such methods comprise determining the DNA sequence
30 of a region of the KCNQ5 gene from the patient and comparing that sequence to the
sequence from the corresponding region of the KCNQ5 gene from a non-affected
person, *i.e.*, a person who does not suffer from Stargardt-like macular dystrophy,
cone-rod dystrophy, Salla disease, or age-related macular degeneration, where a
difference in sequence between the DNA sequence of the KCNQ5 gene from the

patient and the DNA sequence of the KCNQ5 gene from the non-affected person indicates that the patient has Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

Such methods of diagnosis may be carried out in a variety of ways.

5 For example, one embodiment comprises:

- (a) providing PCR primers from a region of the KCNQ5 gene;
- (b) performing PCR on a DNA sample from the patient to produce a PCR fragment from the patient;
- (c) performing PCR on a control DNA sample comprising a
10 nucleotide sequence selected from the group consisting of SEQ.ID.NO:1 and SEQ.ID.NO:2 to produce a control PCR fragment;
- (d) determining the nucleotide sequence of the PCR fragment from the patient and the nucleotide sequence of the control PCR fragment;
- (e) comparing the nucleotide sequence of the PCR fragment from
15 the patient to the nucleotide sequence of the control PCR fragment;

where a difference between the nucleotide sequence of the PCR fragment from the patient and the nucleotide sequence of the control PCR fragment indicates that the patient has a mutation in the KCNQ5 gene and thus is likely to have Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related
20 macular degeneration.

In a particular embodiment, the PCR primers are from a region of the KCNQ5 gene where it is suspected that a patient harbors a mutation. In a particular embodiment, the PCR primers are from the coding region of the KCNQ5 gene, *i.e.*, from the coding region of SEQ.ID.NO:1 or SEQ.ID.NO:2. In a particular
25 embodiment, the PCR primers amplify a region that includes the marker D6S280.

In a particular embodiment, the DNA sample from the patient is cDNA that has been prepared from an RNA sample from the patient. In another embodiment, the DNA sample from the patient is genomic DNA. In a particular embodiment, the control DNA sample is DNA from a person who does not have
30 Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

In a particular embodiment, the nucleotide sequences of the PCR fragment from the patient and the control PCR fragment are determined by DNA sequencing.

In a particular embodiment, the nucleotide sequences of the PCR fragment from the patient and the control PCR fragment are compared by direct comparison after DNA sequencing. In another embodiment, step (d) is omitted and the comparison in step (e) is made by a process that includes hybridizing the PCR
5 fragment from the patient and the control PCR fragment and then using an endonuclease that cleaves at any mismatched positions in the hybrid but does not cleave the hybrid if the two fragments match perfectly. Such an endonuclease is, *e.g.*, S1. In this embodiment, the conversion of the PCR fragment from the patient to smaller fragments after endonuclease treatment indicates that the patient carries a
10 mutation in the KCNQ5 gene. In such embodiments, it may be advantageous to label (radioactively, enzymatically, immunologically, *etc.*) the PCR fragment from the patient or the control PCR fragment.

The present invention provides a method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:

- 15 (a) obtaining an RNA sample from the patient;
- (b) performing reverse transcription-PCR (RT-PCR) on the RNA sample using primers that span a region of the coding sequence of the KCNQ5 gene to produce a PCR fragment from the patient where the PCR fragment from the patient has a defined length, the length being dependent upon the identity of the primers that
20 were used in the RT-PCR;
- (c) hybridizing the PCR fragment to DNA comprising a sequence selected from the group consisting of SEQ.ID.NO:1 and SEQ.ID.NO.:2, or to portions of SEQ.ID.NO:1 or SEQ.ID.NO.:2 that are sufficiently long to give rise to bands that can be seen on polyacrylamide gels, to form a hybrid;
- 25 (d) treating the hybrid produced in step (c) with an endonuclease that cleaves at any mismatched positions in the hybrid but does not cleave the hybrid if the two fragments match perfectly;
- (e) determining whether the endonuclease cleaved the hybrid by determining the length of the PCR fragment from the patient after endonuclease
30 treatment where a reduction in the length of the PCR fragment from the patient after endonuclease treatment indicates that the patient carries a mutation in the KCNQ5 gene.

In a variation of the above-described method, instead of determining the length of the PCR fragment from the patient after endonuclease treatment, the

length of the DNA comprising a sequence selected from the group consisting of SEQ.ID.NO.:1 and SEQ.ID.NO.:2, or the DNA comprising portions of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 that are sufficiently long to give rise to bands that can be seen on polyacrylamide gels is determined after endonuclease treatment. In such a variation, a
5 reduction in the length of the DNA comprising a sequence selected from the group consisting of SEQ.ID.NO.:1 and SEQ.ID.NO.:2, or the DNA comprising portions of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 that are sufficiently long to give rise to bands that can be seen on polyacrylamide gels indicates that the patient carries a mutation in the KCNQ5 gene.

- 10 The present invention provides a method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:
- (a) making cDNA from an RNA sample from the patient;
 - (b) providing a set of PCR primers based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2;
 - 15 (c) performing PCR on the cDNA to produce a PCR fragment from the patient;
 - (d) determining the nucleotide sequence of the PCR fragment from the patient;
 - (e) comparing the nucleotide sequence of the PCR fragment from
20 the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2; where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 indicates that the patient carries a mutation in the KCNQ5 gene.

- The present invention provides a method of diagnosing whether a
25 patient carries a mutation in the KCNQ5 gene that comprises:
- (a) preparing genomic DNA from the patient;
 - (b) providing a set of PCR primers based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2;
 - (c) performing PCR on the genomic DNA to produce a PCR
30 fragment from the patient;
 - (d) determining the nucleotide sequence of the PCR fragment from the patient;
 - (e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2;

where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 indicates that the patient carries a mutation in the KCNQ5 gene.

The present invention also provides oligonucleotide probes, based upon the sequences of SEQ.ID.NO.:1 or SEQ.ID.NO.:2, that can be used in diagnostic methods related to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. In particular, the present invention includes DNA oligonucleotides comprising at least about 10, 15, or 18 contiguous nucleotides of a sequence selected from the group consisting of: SEQ.ID.NO.:1 and SEQ.ID.NO.:2 where the oligonucleotide probe comprises no stretch of contiguous nucleotides longer than 5 of a sequence selected from the group consisting of: SEQ.ID.NO.:1 and SEQ.ID.NO.:2 other than the said at least about 10, 15, or 18 contiguous nucleotides. The oligonucleotides can be substantially free from other nucleic acids. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide probes can be packaged in kits.

In addition to the diagnostic utilities described above, the present invention makes possible the recombinant expression of the KCNQ5 protein in various cell types. Such recombinant expression makes possible the study of this protein so that its biochemical activity and its role in Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration can be elucidated.

The present invention also makes possible the development of assays which measure the biological activity of the KCNQ5 protein. Such assays using recombinantly expressed KCNQ5 protein are especially of interest. Assays for KCNQ5 protein activity can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of KCNQ5 protein. Such identified compounds can serve as "leads" for the development of pharmaceuticals that can be used to treat patients having Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. In versions of the above-described assays, mutant KCNQ5 proteins are used and inhibitors or activators of the activity of the mutant KCNQ5 proteins are identified.

Preferred cell lines for recombinant expression of KCNQ5 are those which do not express endogenous potassium channels (*e.g.*, CV-1, NIH-3T3). Such

cell lines can be loaded with ^{86}Rb , an ion which can pass through potassium channels. The ^{86}Rb -loaded cells can be exposed to collections of substances (*e.g.*, combinatorial libraries, natural products) and those substances that are able to alter ^{86}Rb efflux identified. Such substances are likely to be activators or inhibitors of
5 KCNQ5.

The present invention includes a method of identifying activators or inhibitors of KCNQ5 comprising:

- (a) recombinantly expressing KCNQ5 protein or mutant KCNQ5 protein in a host cell;
- 10 (b) measuring the biological activity of KCNQ5 protein or mutant KCNQ5 protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein;
where a change in the biological activity of the KCNQ5 protein or the mutant KCNQ5 protein in the presence as compared to the absence of the substance
15 indicates that the substance is an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein.

In particular embodiments, the biological activity is the production of a voltage-gated potassium current, or efflux of ^{86}Rb .

In particular embodiments, a vector encoding KCNQ5 is transferred
20 into *Xenopus* oocytes in order to cause the expression of KCNQ5 protein in the oocytes. Alternatively, RNA encoding KCNQ5 protein can be prepared *in vitro* and injected into the oocytes, also resulting in the expression of KCNQ5 protein in the oocytes. Following expression of KCNQ5 in the oocytes, membrane currents are measured after the transmembrane voltage is changed in steps. A change in
25 membrane current is observed when the KCNQ5 channels opens, allowing potassium ion flow. Similar oocytes studies were reported for KCNQ2 and KCNQ3 potassium channels in Wang et al., 1998, Science 282:1890-1893.

Inhibitors of KCNQ5 can be identified by exposing the oocytes expressing KCNQ5 to collections of substances and determining whether the
30 substances can block or diminish the membrane currents observed in the absence of the substance.

Accordingly, the present invention provides a method of identifying inhibitors of KCNQ5 comprising:

- (a) expressing KCNQ5 protein in *Xenopus* oocytes;

(b) changing the transmembrane potential of the oocytes in the presence and the absence of a substance suspected of being an inhibitor of KCNQ5;

(c) measuring membrane potassium currents following step (b);
where if the potassium membrane currents measured in step (c) are
5 greater in the absence rather than in the presence of the substance, then the substance is an inhibitor of KCNQ5.

The present invention also includes assays for the identification of activators and inhibitors of KCNQ5 that are based upon FRET between a first and a second fluorescent dye where the first dye is bound to one side of the plasma
10 membrane of a cell expressing KCNQ5 and the second dye is free to shuttle from one face of the membrane to the other face in response to changes in membrane potential. In certain embodiments, the first dye is impenetrable to the plasma membrane of the cells and is bound predominately to the extracellular surface of the plasma membrane. The second dye is trapped within the plasma membrane but is free to diffuse within
15 the membrane. At normal (*i.e.*, negative) resting potentials of the membrane, the second dye is bound predominately to the inner surface of the extracellular face of the plasma membrane, thus placing the second dye in close proximity to the first dye. This close proximity allows for the generation of a large amount of FRET between the two dyes. Following membrane depolarization, the second dye moves from the
20 extracellular face of the membrane to the intracellular face, thus increasing the distance between the dyes. This increased distance results in a decrease in FRET, with a corresponding increase in fluorescent emission derived from the first dye and a corresponding decrease in the fluorescent emission from the second dye. See figure 1 of González & Tsien, 1997, Chemistry & Biology 4:269-277. See also González &
25 Tsien, 1995, Biophys. J. 69:1272-1280 and U.S. Patent No. 5,661,035.

In certain embodiments, the first dye is a fluorescent lectin or a fluorescent phospholipid that acts as the fluorescent donor. Examples of such a first dye are: a coumarin-labeled phosphatidylethanolamine (*e.g.*, N-(6-chloro-7-hydroxy-2-oxo-2H--1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-
30 ethanolamine) or N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine); a fluorescently-labeled lectin (*e.g.*, fluorescein-labeled wheat germ agglutinin). In certain embodiments, the second dye is an oxonol that acts as the fluorescent acceptor. Examples of such a second dye are: bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols (*e.g.*, bis(1,3-dihexyl-2-

thiobarbiturate)trimethineoxonol) or pentamethineoxonol analogues (*e.g.*, bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; or bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol). See González & Tsien, 1997, Chemistry & Biology 4:269-277 for methods of synthesizing various dyes suitable for use in the present invention. In certain embodiments, the assay may comprise a natural carotenoid, *e.g.*, astaxanthin, in order to reduce photodynamic damage due to singlet oxygen.

The above described assays can be utilized to discover activators and inhibitors of KCNQ5. Such assays will generally utilize cells that express KCNQ5, *e.g.*, by transfection with expression vectors encoding KCNQ5. In assays for inhibitors, such cells will generally have a resting membrane potential that is roughly equal to the threshold for activation of the KCNQ5 channel. This is because most untransfected cells will have membrane potentials that are depolarized relative to the threshold potential of KCNQ5 channels. Therefore, when KCNQ5 is expressed in these cells, the KCNQ5 channels open. This lets K⁺ out of the cells, which tends to hyperpolarize the membrane potential. This closes some of the KCNQ5 channels, leading to relative depolarization. In this way, a steady state develops around the threshold for activation of the KCNQ5 channel. Inhibitors of KCNQ5 will, therefore, disturb this steady state and depolarize the cell. In assays for activators, KCNQ5 will be transfected into a cell line that also expresses a counteracting, depolarizing current. The membrane potential in these cells will therefore be set by contributions of both the KCNQ5 channel and the endogenous depolarizing current, resulting in a more depolarized resting potential. Ideally, the endogenous current will play the major role in the absence of a KCNQ5 activator. Activators of KCNQ5 will open this channel and increase the contribution of KCNQ5 to the membrane potential relative to the other current and the potential will, therefore, hyperpolarize in response to an activator of KCNQ5. Changes in membrane potential (depolarizations and hyperpolarizations) that are caused by activators and inhibitors of KCNQ5 can be monitored by the assays using FRET described above.

Accordingly, the present invention provides a method of identifying activators of KCNQ5 comprising:

- (a) providing test cells comprising:
 - (1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an activator of KCNQ5;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is greater than the amount of FRET exhibited by the control cells, the substance is an activator of KCNQ5;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

The present invention also provides a method of identifying inhibitors of KCNQ5 comprising:

(a) providing test cells comprising:

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

(3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;

(b) exposing the test cells to a substance that is suspected of being an inhibitor of KCNQ5;

(c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

(d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is less than
5 the amount of FRET exhibited by the control cells, the substance is an inhibitor of KCNQ5;

where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been
10 exposed to the substance.

In a variation of the assay described above, instead of the transfected cell's membrane potential being allowed to reach steady state on its own, the membrane potential is artificially set at a potential in which the KCNQ5 channel is open. This can be done, *e.g.*, by variation of the external K^+ concentration in a
15 known manner (*e.g.*, increased concentrations of external K^+). If such cells, having open KCNQ5 channels, are exposed to inhibitors of KCNQ5, the KCNQ5 channels will close, and the cells' membrane potentials will be depolarized. This depolarization can be observed as a decrease in FRET.

Accordingly, the present invention provides a method of identifying
20 inhibitors of KCNQ5 comprising:

- (a) providing cells comprising:
 - (1) an expression vector that directs the expression of KCNQ5 in the cells;
 - (2) a first fluorescent dye, where the first dye is bound to
25 one side of the plasma membrane; and
 - (3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;
- (b) adjusting the membrane potential of the cells such that the ion
30 channel formed by KCNQ5 is open;
- (c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells;
- (d) repeating step (b) and step (c) while the cells are exposed to a substance that is suspected of being an inhibitor of KCNQ5;

where if the amount of FRET exhibited by the cells that are exposed to the substance is less than the amount of FRET exhibited by the cells that have not been exposed to the substance, then the substance is an inhibitor of KCNQ5.

In particular embodiments of the above-described methods, the
5 expression vectors are transfected into the test cells.

In particular embodiments of the above-described methods, KCNQ5 has an amino acid sequence of SEQ.ID.NO.:3.

In particular embodiments of the above-described methods, the first
10 fluorescent dye is selected from the group consisting of: a fluorescent lectin; a fluorescent phospholipid; a coumarin-labeled phosphatidylethanolamine; N-(6-chloro-7-hydroxy-2-oxo-2H--1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidylethanolamine); N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine); and fluorescein-labeled wheat germ agglutinin.

15 In particular embodiments of the above-described methods, the second fluorescent dye is selected from the group consisting of: an oxonol that acts as the fluorescent acceptor; bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)trimethineoxonol; bis(1,3-dialkyl-2-thiobarbiturate)quatramethineoxonols; bis(1,3-dialkyl-2-
20 thiobarbiturate)pentamethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol; and bis(1,3-dialkyl-2-thiobarbiturate)hexamethineoxonols.

In a particular embodiment of the above-described methods, the cells
25 are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I
30 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* melanophores, or *Xenopus* oocytes.

In particular embodiments of the above-described methods, the control cells do not comprise item (a)(1) but do comprise items (a)(2) and (a)(3).

In assays to identify activators or inhibitors of KCNQ5, it may be advantageous to co-express another potassium channel, *e.g.*, KCNQ1, KCNQ2, KCNQ3, or KCNQ4, together with KCNQ5, or with an accessory subunit, such as the Isk protein or one of its homologues, in order to form a functional heteromeric potassium channel.

While the above-described methods are explicitly directed to testing whether "a" substance is an activator or inhibitor of KCNQ5, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of KCNQ5. Accordingly, the use of collections of substances, or individual members of such collections, as the substance in the above-described methods is within the scope of the present invention.

The present invention includes pharmaceutical compositions comprising activators or inhibitors of KCNQ5 protein that have been identified by the herein-described methods. The activators or inhibitors are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the activators or inhibitors.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where KCNQ5 activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, gender, and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion),

intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or
5 four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the
10 dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and
15 the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to
20 target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

The present invention includes a method of treating Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, age-related macular degeneration and other forms of macular degeneration, deafness, epilepsy, and
25 different forms of neuropsychiatric, heart, gastrointestinal, and muscle disorders by administering to a patient a therapeutically effective amount of a substance that is an activator or an inhibitor of a voltage-gated potassium channel containing the KCNQ5 protein.

When screening compounds in order to identify potential
30 pharmaceuticals that specifically interact with a target ion channel, it is necessary to ensure that the compounds identified are as specific as possible for the target ion channel. To do this, it is necessary to screen the compounds against as wide an array as possible of ion channels that are similar to the target ion channel. Thus, in order to find compounds that are potential pharmaceuticals that interact with ion channel A, it

is not enough to ensure that the compounds interact with ion channel A (the "plus target") and produce the desired pharmacological effect through ion channel A. It is also necessary to determine that the compounds do not interact with ion channels B, C, D, *etc.* (the "minus targets"). In general, as part of a screening program, it is
5 important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). KCNQ5 protein, DNA encoding KCNQ5 protein, and recombinant cells that have been engineered to express KCNQ5 protein have utility in that they can be used as "minus targets" in screens designed to identify compounds that specifically interact with other ion channels. For example, Wang et
10 al., 1998, Science 282:1890-1893 have shown that KCNQ2 and KCNQ3 form a heteromeric potassium ion channel know as the "M-channel." The M-channel is an important target for drug discovery since mutations in KCNQ2 and KCNQ3 are responsible for causing epilepsy (Biervert et al., 1998, Science 279:403-406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690). A
15 screening program designed to identify activators or inhibitors of the M-channel would benefit greatly by the use of KCNQ5 as a minus target.

The present invention also includes antibodies to the KCNQ5 protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire KCNQ5 protein or
20 against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-
25 186.

For the production of polyclonal antibodies, KCNQ5 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of
30 antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, KCNQ5 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case

- of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

- Gene therapy may be used to introduce KCNQ5 polypeptides into the cells of target organs, *e.g.*, the pigmented epithelium of the retina or other parts of the retina. Nucleotides encoding KCNQ5 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, lentivirus, and polio virus based vectors. Alternatively, nucleotides encoding KCNQ5 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with KCNQ5 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate KCNQ5 activity.

- The present invention includes processes for cloning orthologues of human KCNQ5 from non-human species. In general, such processes include preparing a PCR primer or a hybridization probe based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2 that can be used to amplify a fragment containing the non-human KCNQ5 (in the case of PCR) from a suitable DNA preparation or to select a cDNA or genomic clone containing the non-human KCNQ5 from a suitable library. A preferred embodiment of this process is a process for cloning the KCNQ5 gene from mouse.

- By providing DNA encoding mouse KCNQ5, the present invention allows for the generation of an animal model of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration. Such animal models can be generated by making transgenic "knockout" or "knockin" mice containing altered KCNQ5 genes. Knockout mice can be generated in which portions of the mouse KCNQ5 gene have been deleted. Knockin mice can be generated in which mutations that have been shown to lead to Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration when present in the human KCNQ5 gene are introduced into the mouse gene. Such knockout and

knockin mice will be valuable tools in the study of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration and will provide important model systems in which to test potential pharmaceuticals or treatments for Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

Accordingly, the present invention includes a method of producing a mouse model of Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration comprising:

- (a) designing PCR primers or an oligonucleotide probe based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2 for use in cloning the mouse KCNQ5 gene;
- (b) using the PCR primers or the oligonucleotide probe to clone at least a portion of the mouse KCNQ5 gene, the portion being large enough to use in making a transgenic mouse;
- (c) producing a transgenic mouse having at least one copy of the mouse KCNQ5 gene altered from its native state.

Methods of producing knockout and knockin mice are well known in the art. One method involves the use of gene-targeted ES cells in the generation of gene-targeted transgenic knockout mice and is described in, *e.g.*, Thomas et al., 1987, Cell 51:503-512, and is reviewed elsewhere (Frohman et al., 1989, Cell 56:145-147; Capecchi, 1989, Trends in Genet. 5:70-76; Baribault et al., 1989, Mol. Biol. Med. 6:481-492).

Techniques are available to inactivate or alter any genetic region to virtually any mutation desired by using targeted homologous recombination to insert specific changes into chromosomal genes. Generally, use is made of a "targeting vector," *i.e.*, a plasmid containing part of the genetic region it is desired to mutate. By virtue of the homology between this part of the genetic region on the plasmid and the corresponding genetic region on the chromosome, homologous recombination can be used to insert the plasmid into the genetic region, thus disrupting the genetic region. Usually, the targeting vector contains a selectable marker gene as well.

In comparison with homologous extrachromosomal recombination, which occurs at frequencies approaching 100%, homologous plasmid-chromosome recombination was originally reported to only be detected at frequencies between 10^{-6} and 10^{-3} (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395; Smithies et al., 1985, Nature 317: 230-234; Thomas et al., 1986, Cell 44:419-428).

Nonhomologous plasmid-chromosome interactions are more frequent, occurring at levels 10^5 -fold (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395) to 10^2 -fold (Thomas et al., 1986, Cell 44:419-428) greater than comparable homologous insertion.

5 To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting homologous alteration events uses the polymerase chain reaction (PCR) to screen pools of transformant cells for homologous insertion, followed by screening individual clones (Kim et al., 1988, Nucleic Acids Res. 16:8887-8903; Kim et al., 1991, Gene 103:227-233).
10 Alternatively, a positive genetic selection approach has been developed in which a marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly (Sedivy et al., 1989, Proc. Natl. Acad. Sci. USA 86:227-231). One of the most powerful approaches developed for
15 selecting homologous recombinants is the positive-negative selection (PNS) method developed for genes for which no direct selection of the alteration exists (Mansour et al., 1988, Nature 336:348-352; Capecchi, 1989, Science 244:1288-1292; Capecchi, 1989, Trends in Genet. 5:70-76). The PNS method is more efficient for targeting genes which are not expressed at high levels because the marker gene has its own
20 promoter. Nonhomologous recombinants are selected against by using the Herpes Simplex virus thymidine kinase (HSV-TK) gene and selecting against its nonhomologous insertion with herpes drugs such as gancyclovir (GANC) or FIAU (1-(2-deoxy 2-fluoro-B-D-arabinofluranosyl)-5-iodouracil). By this counter-selection, the percentage of homologous recombinants in the surviving transformants can be
25 increased.

Other methods of producing transgenic mice involve microinjecting the male pronuclei of fertilized eggs. Such methods are well known in the art.

30 The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Identification of the human KCNQ5 gene and cDNA cloningConstruction of Libraries for Shotgun Sequencing from PAC Clones

Bacterial strains containing the KCNQ5 PACs (P1 Artificial Chromosomes) were received from Research Genetics (Huntsville, AL). Cells were streaked on Luria-Bertani (LB) agar plates supplemented with the appropriate antibiotic. A single colony was used to prepare a 5-ml starter culture and then 1-L overnight culture in LB medium. The cells were pelleted by centrifugation and PAC DNA was purified by equilibrium centrifugation in cesium chloride-ethidium bromide gradient (Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press). Purified PAC DNA was brought to 50 mM Tris pH 8.0, 15 mM MgCl₂, and 25% glycerol in a volume of 2 ml and placed in a AERO-MIST nebulizer (CIS-US, Bedford, MA). The nebulizer was attached to a nitrogen gas source and the DNA was randomly sheared at 10 psi for 30 sec. The sheared DNA was ethanol precipitated and resuspended in TE (10 mM Tris, 1 mM EDTA). The ends were made blunt by treatment with Mung Bean Nuclease (Promega, Madison, WI) at 30°C for 30 min, followed by phenol/chloroform extraction, and treatment with T4 DNA polymerase (GIBCO/BRL, Gaithersburg, MD) in multicore buffer (Promega, Madison, WI) in the presence of 40 uM dNTPs at 16°C. To facilitate subcloning of the DNA fragments, BstX I adapters (Invitrogen, Carlsbad, CA) were ligated to the fragments at 14°C overnight with T4 DNA ligase (Promega, Madison, WI). Adapters and DNA fragments less than 500 bp were removed by column chromatography using a cDNA sizing column (GIBCO/BRL, Gaithersburg, MD) according to the instructions provided by the manufacturer. Fractions containing DNA greater than 1 kb were pooled and concentrated by ethanol precipitation. The DNA fragments containing BstX I adapters were ligated into the BstX I sites of pSHOT II which was constructed by subcloning the BstX I sites from pcDNA II (Invitrogen, Carlsbad, CA) into the BssH II sites of pBlueScript (Stratagene, La Jolla, CA). pSHOT II was prepared by digestion with BstX I restriction endonuclease and purified by agarose gel electrophoresis. The gel purified vector DNA was extracted from the agarose by following the Prep-A-Gene (BioRad, Richmond, CA) protocol. To reduce ligation of

the vector to itself, the digested vector was treated with calf intestinal phosphatase (GIBCO/BRL, Gaithersburg, MD). Ligation reactions of the DNA fragments with the cloning vector were transformed into ultra-competent XL-2 Blue cells (Stratagene, La Jolla, CA), and plated on LB agar plates supplemented with 100 µg/ml ampicillin.

- 5 Individual colonies were picked into a 96 well plate containing 100 µl/well of LB broth supplemented with ampicillin and grown overnight at 37°C. Approximately 25 µl of 80% sterile glycerol was added to each well and the cultures stored at -80°C.

Preparation of plasmid DNA

- 10 Glycerol stocks were used to inoculate 5 ml of LB broth supplemented with 100 µg/ml ampicillin either manually or by using a Tecan Genesis RSP 150 robot (Tecan AG, Hombrechtikon, Switzerland) programmed to inoculate 96 tubes containing 5 ml broth from the 96 wells. The cultures were grown overnight at 37°C with shaking to provide aeration. Bacterial cells were pelleted by centrifugation, the
15 supernatant decanted, and the cell pellet stored at -20°C. Plasmid DNA was prepared with a QIAGEN Bio Robot 9600 (QIAGEN, Chatsworth, CA) according to the Qiawell Ultra protocol. To test the frequency and size of inserts, plasmid DNA was digested with the restriction endonuclease Pvu II. The size of the restriction endonuclease products was examined by agarose gel electrophoresis with the average
20 insert size being 1 to 2 kb.

DNA Sequence Analysis of Shotgun clones

- DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS
25 (Perkin Elmer, Norwalk, CT). DNA sequence analysis was performed with M13 forward and reverse primers. Following amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). Approximately 4 sequencing reactions were performed per kb of DNA to be examined (384 sequencing reactions per each of nine
30 PACs).

Assembly of DNA sequences

Phred/Phrap was used for DNA sequences assembly. This program was developed by Dr. Phil Green and licensed from the University of Washington

(Seattle, WA). Phred/Phrap consists of the following programs: Phred for base-calling, Phrap for sequence assembly, Crossmatch for sequence comparisons, Consed and Phrapview for visualization of data, Repeatmasker for screening repetitive sequences. Vector and *E. coli* DNA sequences were identified by Crossmatch and removed from the DNA sequence assembly process. DNA sequence assembly was on a SUN Enterprise 4000 server running a Solaris 2.51 operating system (Sun Microsystems Inc., Mountain View, CA) using default Phrap parameters. The sequence assemblies were further analyzed using Consed and Phrapview.

10 Genomic sequence of the KCNQ5 gene and its exon/intron organization

Genomic DNA sequence from PAC 141B1 was compared with GenBank database entries using the BLASTN and BLASTX algorithms of the AceDB package. This comparison originally revealed a total of 5 exons (exons 3(D), 4 (A), 5(B), 6(E), and 7(C) delineated in Figure1), based on their homology to the known potassium channel genes KCNQ1, KCNQ2, KCNQ3, and KCNQ4. Full-length cDNA was rescued from the pools of the human fetal brain cDNA library using the RCCA technique described in Example 2. Comparison of the cDNA sequence and genomic sequence of PAC141B1 revealed a total of 8 exons (exons 3-10 delineated in Figure1). Genomic regions corresponding to exons 1,2, and 11-14 were not present in PAC141B1.

In order to identify the genomic region corresponding to exon 2 and its right flanking intron, oligonucleotide KCN-2L2 (TTTTCTCCTTGTCTTTGGTTGCTTG; SEQ.ID.NO.:11) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-2L1 (CCTCAAGTTGCCTCTTGATCCTG; SEQ.ID.NO.:13) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 2 and its left flanking intron, oligonucleotide KCN-2R1 (CAGGATCAAGAGGCAACTTGAGG; SEQ.ID.NO.:15) from the KCNQ5 cDNA in combination with the adaptor primer AP1

(CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-2R2 (CCAATTTTGTGTGCTCAGGGATGGTAGA; SEQ.ID.NO.:16) in
 5 combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 11 and its right flanking intron, oligonucleotide KCN-11L1 (GACACAGCCCTTGGCACT; SEQ.ID.NO.:17) from the KCNQ5 cDNA in combination with the adaptor primer
 10 AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-11L2 (GATGATGTATATGATGAAAAAGGATG; SEQ.ID.NO.:18) in combination with the nested adaptor primer AP2
 15 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 11 and its left flanking intron, oligonucleotide KCN-11R1 (CTGATAGCTCGAATGACAGTTTT; SEQ.ID.NO.:19) from the KCNQ5 cDNA in combination with the adaptor primer AP1
 20 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN11-R2 (AAGTGGTGGGGTGAGGTCTTCCACTG; SEQ.ID.NO.:20) in combination with the nested adaptor primer AP2
 25 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 12 and its right flanking intron, oligonucleotide KCN-12L1 (AGA ATT ATG AAA TTT CAT GTT GCA; SEQ.ID.NO.:21) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12)
 30 was used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-12L2 (AAA CGG AAG TTT AAG GAA ACA TT; SEQ.ID.NO.:22) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

In order to identify the genomic region corresponding to exon 12 and its left flanking intron, oligonucleotide KCN-12R1 (ACG TGT TTG TTG GCT TTT AAT TC; SEQ.ID.NO.:23) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was
5 used to PCR-amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-12R2 (TAC ACA ACA TGT CCA GAT GAC; SEQ.ID.NO.:24) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

10 In order to identify the genomic region corresponding to exon 13 and its right flanking intron, oligonucleotide KCN-13L1 (TGATCAAATTCTTGGAAGGG; SEQ.ID.NO.:25) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-
15 amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-13L2 (TCACATCAGATAAGAAGAGCCGA; SEQ.ID.NO.:26) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

20 In order to identify the genomic region corresponding to exon 13 and its left flanking intron, oligonucleotide KCN-13R1 (GTTTTTCAACCTTGACCACCC; SEQ.ID.NO.:27) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-
25 amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-13R2 (AGCATACTGAGATCGTCTGTGGT; SEQ.ID.NO.:28) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

30 In order to identify the genomic region corresponding to exon 14 and its left flanking intron, oligonucleotide KCN-2543R(AATTCCAAAAGTGTCTGTCTCTGTC; SEQ.ID.NO.:29) from the KCNQ5 cDNA in combination with the adaptor primer AP1 (CCATCCTAATACGACTCACTATAGGGC; SEQ.ID.NO.:12) was used to PCR-

amplify the DNA from a GenomeWalker kit purchased from Clontech (Palo Alto, CA). Diluted PCR product was subjected to PCR amplification with nested primer KCN-2512R (GGACCCACCTCTTCATCAGTTA; SEQ.ID.NO.:30) in combination with the nested adaptor primer AP2 (ACTCACTATAGGGCTCGAGCGGC; SEQ.ID.NO.:14).

Products obtained from these PCR amplifications were analyzed using ABI 377 sequencers according to standard protocols. Comparison of the full-length KCNQ5 cDNA sequence with the sequences of PAC141B1 and sequences obtained in PCR reactions with DNA from the GenomeWalker kit revealed all 14 exons of the KCNQ5 gene. Exact sequence of exon/intron boundaries within the KCNQ5 gene were determined for exons 2-14. The splice signals in all introns conform to published consensus sequences.

EXAMPLE 2

15 Cloning of KCNQ5 cDNA

The DNA sequence of the cDNA fragment that matches exons 3(D), 4 (A), 5(B), 6(E), and 7(C) of the KCNQ5 was deduced from the genomic sequence of PAC 141B1. Subsequent sequencing of PCR fragments obtained in RCCA reactions confirmed the presence of this fragment in the cDNA library from human fetal brain. This original cDNA fragment corresponds to the cDNA region with coordinates 368-1,004 in Figure 2.

A PCR based technique termed Reduced Complexity cDNA Analysis (RCCA) was used to extend this original cDNA fragment. RCCA is similar to procedures reported by Munroe et al., 1995, Proc. Natl. Acad. Sci. USA 92: 2209-2213 and Wilfinger et al., 1997, BioTechniques 22:481-486 and relies upon a PCR template that is a pool of approximately 20,000 cDNA clones; this reduces the complexity of the template and increases the probability of obtaining longer PCR extensions.

96 wells of a human fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 483 bp PCR product using primers KCN-DL (GGAAGACTGAGGTTTGCTCG; SEQ.ID.NO.31) and KCN-ER (GGCAGGAAGTGCAAAGAAAG; SEQ.ID.NO.32). Eight wells were found to

contain the correct 483 bp fragment by PCR analysis. 5' and 3' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primers KCN-DL and KCN-ER because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primers KCN-EL (CTTTCTTTGCACTTCCTGCC; SEQ.ID.NO.37) and KCN-DR1 (AACACAGAAGGGCTTTCGAG; SEQ.ID.NO.38). The PCR products were separated from the unincorporated dNTP's and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols.

PCR fragments were assembled into a contig termed "KCN consensus 2_16_99" that corresponds to the cDNA region with coordinates 278-1,456 in Figure 2. A second round of the RCCA analysis was performed to obtain the clones extending to the 3' end of the cDNA contig termed "KCN consensus 2_16_99". 96 wells of a human fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 117 bp PCR product using primers KCN-11L1 (GACACAGCCCTTGGCACT; SEQ.ID.NO.17) and KCN-11R1 (CTGATAGCTCGAATGACAGTTTT; SEQ.ID.NO.19) that were derived from the 3' sequence of the cDNA contig termed "KCN consensus 2_16_99". A number of wells were found to contain the correct 117 bp fragment by PCR analysis. 3' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primer KCN-11L1 (GACACAGCCCTTGGCACT; SEQ.ID.NO.17) because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primer

KCN11-R2 (AAGTGGTGGGGTGAGGTCTTCCACTG; SEQ.ID.NO.20). The PCR products were separated from the unincorporated dNTPs and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols.

5 PCR fragments were assembled into a contig termed "KCN consensus 2_26_99" that corresponds to the cDNA region with coordinates 278-2,527 in Figure 2. A third round of RCCA analysis was performed to obtain the clones extending to the 5' end of the cDNA contig termed "KCN consensus 2_26_99". 96 wells of a human fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 214 bp PCR product using primers KCN-2L2 (TTTTCTCCTTGTCTTTGGTTGCTTG; SEQ.ID.NO.11) and KCN-DR1 (AACACAGAAGGGCTTTCGAG; SEQ.ID.NO.38) that were derived from the 5' sequence of the cDNA contig termed "KCN consensus 2_26_99". A number of wells were found to contain the correct 214 bp fragment by PCR analysis. 5' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primer KCN-DR1 (AACACAGAAGGGCTTTCGAG; SEQ.ID.NO.38) because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primer KCN-DR2 (CAGTCTTCCTTGCCATCCTC; SEQ.ID.NO.39). The PCR products were separated from the unincorporated dNTPs and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols.

30 PCR fragments were assembled into a contig termed "KCN consensus 3_3_99" that corresponds to the cDNA region with coordinates 1-2,527 in Figure 2. A fourth round of RCCA analysis was performed to obtain the clones extending to the 3' end of the cDNA contig termed "KCN consensus 3_3_99". 96 wells of a human

fetal brain plasmid library were scanned, 20,000 clones per well, by amplifying a 145 bp PCR product using primers KCN-2106L (GCAGCCCCAACAACCTTTACA; SEQ.ID.NO.40) and KCN-2250R (CATTTTCCTTGGAGGCAACA; SEQ.ID.NO.41) that were derived from the 3' sequence of the cDNA contig termed "KCN consensus 3_3_99". A number of wells were found to contain the correct 214 bp fragment by PCR analysis. 5' RACE was subsequently performed on the positive wells containing the plasmid cDNA library using a vector specific primer and a gene specific primer. The vector specific primers, PBS 543R (GGGGATGTGCTGCAAGGCGA; SEQ.ID.NO.33) and PBS 873F (CCCAGGCTTTACACTTTATGCTTCC; SEQ.ID.NO.34) were both used in combination with gene specific primer KCN-2106L (GCAGCCCCAACAACCTTTACA; SEQ.ID.NO.40) because the orientation of the insert was not known. After the initial PCR amplification, a nested PCR reaction was performed using nested vector primers PBS 578R (CCAGGGTTTCCCAGTCACGAC; SEQ.ID.NO.35) and PBS 838F (TTGTGTGGAATTGTGAGCGGATAAC; SEQ.ID.NO.36) and gene specific primer KCN-2165L (GCCAGAACTCTGCACCCTA; SEQ.ID.NO.42). The PCR products were separated from the unincorporated dNTP's and primers using Qiagen, QIAquick PCR purification spin columns using standard protocols and resuspended in 30 µl of water. The products were analyzed on ABI 377 sequencers according to standard protocols; PCR fragments were assembled into a contig termed "KCN consensus 3_15_99" that corresponds to the cDNA sequence depicted in Figure 2.

EXAMPLE 3

25 Analysis of expression of KCNQ5

RT-PCR: RT-PCR experiments were performed on "quick-clone" human cDNA samples available from Clontech, Palo Alto, CA. cDNA samples from heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and retina were amplified with primers KCN-DL (GGAAGACTGAGGTTTGCTCG; SEQ.ID.NO.31) and KCN-ER (GGCAGGAAGTGCAAAGAAAG; SEQ.ID.NO.32) in the following PCR conditions:

1. 94°C 10 min
2. 94°C 30 sec
3. 72°C 2 min (decrease this temperature by 1.1°C per cycle)
4. 72°C 2 min
- 5 5. Go to step 2 21 more times
6. 94°C 30 sec
7. 55°C 2 min
8. 72°C 2 min
9. Go to step 6 19 more times
- 10 10. 72°C 7 min
11. 4°C

The KCNQ5 gene was found to be predominantly expressed in human retina and brain (Figure 3B).

15

- Northern blot analysis:* Northern blots containing poly(A+)-RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas were purchased from Clontech, Palo Alto, CA. Primers KCN-DL (GGAAGACTGAGGTTTGCTCG; SEQ.ID.NO.31) and KCN-ER (GGCAGGAAGTGCAAAGAAAG; SEQ.ID.NO.32) were used to amplify a PCR product of 483 bp from a quick-clone human retina cDNA available from Clontech, Palo Alto, CA. This fragment was purified on an agarose gel, the DNA extracted and used as a probe for Northern blot hybridization.

- The probe was labeled by random priming with the Amersham Rediprime kit (Arlington Heights, IL) in the presence of 50-100 μ Ci of 3000 Ci/mmol [alpha 32 P]dCTP (Dupont/NEN, Boston, MA). Unincorporated nucleotides were removed with a ProbeQuant G-50 spin column (Pharmacia/Biotech, Piscataway, NJ). The radiolabeled probe at a concentration of greater than 1×10^6 cpm/ml in rapid hybridization buffer (Clontech, Palo Alto, CA) was incubated overnight at 65°C. The blots were washed by two 15 min incubations in 2X SSC, 0.1% SDS (prepared from 20X SSC and 20 % SDS stock solutions, Fisher, Pittsburgh, PA) at room temperature, followed by two 15 min incubations in 1X SSC, 0.1% SDS at room temperature, and two 30 min incubations in 0.1X SSC, 0.1% SDS

at 60°C. Autoradiography of the blots was done to visualize the bands that specifically hybridized to the radiolabeled probe.

The probe hybridized to an mRNA transcript that is predominately expressed in brain and retina (Figure 3A).

5

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

10

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. An isolated DNA comprising nucleotides encoding a KCNQ5 protein.
5
2. The DNA of claim 1 comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:3.
3. The DNA of claim 1 comprising a nucleotide sequence
10 selected from the group consisting of: SEQ.ID.NO.:1, SEQ.ID.NO.:2, and positions 138-2,675 of SEQ.ID.NO.:2.
4. An isolated DNA that hybridizes under stringent conditions to a nucleotide sequence selected from the group consisting of: SEQ.ID.NO.:1 and
15 SEQ.ID.NO.:2.
5. An expression vector comprising the DNA of claim 1.
6. A recombinant host cell comprising the DNA of claim 1.
20
7. An isolated KCNQ5 protein.
8. The KCNQ5 protein of claim 7 having the amino acid sequence
25 SEQ.ID.NO.: 3.
9. The KCNQ5 protein of claim 8 containing a single amino acid substitution.
10. The KCNQ5 protein of claim 8 containing two or more amino
30 acid substitutions where the amino acid substitutions do not occur in a position where the amino acid substituted in KCNQ5 is also present in the corresponding position of any one of KCNQ2, KCNQ3, or KCNQ4.

11. An antibody that binds specifically to a KCNQ5 protein where the KCNQ5 protein has the amino acid sequence SEQ.ID.NO.:3.

12. A method of diagnosing whether a patient has Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration that comprises determining the DNA sequence of a region of the KCNQ5 gene from the patient and comparing that sequence to the sequence from the corresponding region of the KCNQ5 gene from a non-affected person, *i.e.*, a person who does not have Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration, where a difference in sequence between the DNA sequence of the KCNQ5 gene from the patient and the DNA sequence of the KCNQ5 gene from the non-affected person indicates that the patient has Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, or age-related macular degeneration.

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13. A method of diagnosing whether a patient carries a mutation in the KCNQ5 gene that comprises:

- (a) providing a DNA sample from the patient;
- (b) providing a set of PCR primers based upon SEQ.ID.NO.:1 or SEQ.ID.NO.:2;
- (c) performing PCR on the DNA sample to produce a PCR fragment from the patient;
- (d) determining the nucleotide sequence of the PCR fragment from the patient;
- (e) comparing the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2; where a difference between the nucleotide sequence of the PCR fragment from the patient with the nucleotide sequence of SEQ.ID.NO.:1 or SEQ.ID.NO.:2 indicates that the patient carries a mutation in the KCNQ5 gene.

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14. The method of claim 13 where the DNA sample is genomic DNA.

15. The method of claim 13 where the DNA sample is cDNA.

16. A DNA or RNA oligonucleotide probe comprising at least 18 contiguous nucleotides of at least one of a sequence selected from the group consisting of: SEQ.ID.NO.:1 and SEQ.ID.NO.:2.

5

17. A method for determining whether a substance is an activator or an inhibitor of a KCNQ5 protein or a mutant KCNQ5 protein comprising:

(a) recombinantly expressing KCNQ5 protein or mutant KCNQ5 protein in a host cell;

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(b) measuring the biological activity of KCNQ5 protein or mutant KCNQ5 protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein;

where a change in the biological activity of the KCNQ5 protein or the mutant KCNQ5 protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of KCNQ5 protein or mutant KCNQ5 protein.

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18. A method of identifying inhibitors of KCNQ5 comprising:

(a) expressing KCNQ5 protein in *Xenopus* oocytes;

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(b) changing the transmembrane potential of the oocytes in the presence and the absence of a substance suspected of being an inhibitor of KCNQ5;

(c) measuring membrane potassium currents following step (b);

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where if the potassium membrane currents measured in step (c) are greater in the absence rather than in the presence of the substance, then the substance is an inhibitor of KCNQ5.

19. A method of identifying activators of KCNQ5 comprising:

(a) providing test cells comprising:

30

(1) an expression vector that directs the expression of KCNQ5 in the cells;

(2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and

- (3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;
- (b) exposing the test cells to a substance that is suspected of being an activator of KCNQ5;
- (c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;
- (d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;
- wherein if the amount of FRET exhibited by the test cells is greater than the amount of FRET exhibited by the control cells, the substance is an activator of KCNQ5;
- where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

20. A method of identifying inhibitors of KCNQ5 comprising:
- (a) providing test cells comprising:
- (1) an expression vector that directs the expression of KCNQ5 in the cells;
- (2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane; and
- (3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane to the other face in response to changes in membrane potential;
- (b) exposing the test cells to a substance that is suspected of being an inhibitor of KCNQ5;
- (c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;
- (d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

wherein if the amount of FRET exhibited by the test cells is less than the amount of FRET exhibited by the control cells, the substance is an inhibitor of KCNQ5;

- 5 where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

- 10 21. A method of treating Stargardt-like macular dystrophy, cone-rod dystrophy, Salla disease, age-related macular degeneration, other forms of macular degeneration, deafness, epilepsy, different forms of neuropsychiatric, heart, gastrointestinal, and muscle disorders by administering to a patient a therapeutically effective amount of a substance that is an activator or an inhibitor of a voltage-gated potassium channel containing the KCNQ5 protein.

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FIGURE 1A

KCN6q gene: DNA sequence

1. Underlined nucleotides in capitals represent exons.
2. Initiating ATG codon in exon 1 and terminating TAA codon in exon 14 are shown in bold italics
3. D6D280 genetic marker and phosphoglycerate mutase pseudogene are bold underlined
4. The exact lengths of the gaps between exons 1 and 2, 2 and 3, 10 and 11, 11 and 12, 12 and 13, 13 and 14 are unknown; these gaps are presented as runs of ten **bold n** as a convenience only

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1  CTGGAGTGAG GCGCGGGAAG ATGCCTGGTC CTGCGCTCGC GGACTTGGCA
51 GCCGCGTCTT GCGGGTCTGT CCACTGAACT GCTGAGGACT GCGGCGGTGG
101 CCTGAGGGAG AGCCGCCGGG GCAAGCAGGG GCGCCGGATG AGCCTGCTGG
151 GGAAGCCGCT CTCTTACACG AGTAGCCAGA GCTGCCGGCG CAACGTCAAG
201 TACCGGCGGG TGCAGAACTA CCTGTACAAC GTGCTGGAGA GACCCGCGG
251 CTGGGCGTTC ATCTACCACG CTTTCGTnnn nnnnnnnttc ctttctatt
301 cttattatta atatatgac ttattattaa taatataaag gaatagcaaa
351 tgagaatcca tgagcaatat cagaccatga aaatgagcca gtggctgagt
401 aacaaccaat taggacactt gatagtttag caaagttgcc aaacaggaga
451 cagactcggc tcctttgaac gaagagtgc tgcaagtgtg attccccaga
501 taggagagca agaacatac ttctgggcct ctctcaggat cgttgtttgg
551 gaaggaagtt gtatgggaaa ttcacaaact cttagatgct aacattttaa
601 tgcagcatgc cacacacaca aaccacaaaa cacaaccttt tttcatcaa
651 taaaattgca gaggagcccc atttgcacag tatatcacat tgtattttaa
701 tatccaaaat ggctagtccc ttccagagtt tttatgagtt aatgtgtgct
751 aatttaattg gcctggtgct ttattcattt gaagcaagaa attaagtctg
801 tgataataag gtaaggttct tatcagattt ctctttttgt tgttttacag
851 TTTTCTCCTT GTCTTTGGTT GCTTGATTTT GTCAGTGTTC TCTACCATCC
901 CTGAGCACAC AAAATTGGCC TCAAGTTGCC TCTTGATCCT Ggtaagtga
951 acatgaacaa gaacgtacat gaatgttgta taagaactgc ctataacatt
1001 tatactatgc atcttatcct acaaaaaaat cctatctaaa aaagagttac
1051 tgagaaatat aaaaatgtca aagattactg aaacatttgc ccaccaattt
1101 aacatgtagt caatccttag aaatatatag aaatgttcag gattgctatt
1151 acacagcaat atcttgtgtt gtagatatat cataaataga aggcaatatt
1201 agaaagcagt tttaaagtatg tttatctatg ctaataaaca aattatataa
1251 gaagaatcag tatctatgag gcctctcatt atattgtgaa agactataga
1301 gtagagagca ttttccaata actgtaattt ggcagtagct aaatataatt
1351 ggccaagaac tatgaacata tggcacctca taagaaaata gaaggctcct
1401 tcatgctctt ttcaaccaac agactgcatt atgagttttg ctgctaattg
1451 agttacctgg tgataaattc tgcagtttgc tctgtttcca ttatgctgtc
1501 aatcctcaac cacacagaat tgctcaattc actttnnnnn nnnnnacgag
1551 gtcaggaggt cgagaccatc ctgcctaaca cggtgaaacc ccgcctctac
1601 taaaaataca aaaaattagc tgggcgtagc tactcaggag gctgaggcag
1651 gagaatggcg tgaacctggg aggcggagct tgcagtgcgc cgagattgcg
1701 tcaactgcact ccagcgacag agccagactc cgtctcaaaa aaaaaaaaaa
1751 aaaaaaaaaa gagtatactt gatttatggc atgagtgggtc ttgaatgatt
1801 ttgatggatg actggaaca attagagata taaataaata gcacagaatc
1851 atgacagatt tcatgaagaa tacactgtga agattcacat ggttaataac
1901 attgaaatta ttaaaataaa agagactgca tatattagat tttctttgtg
1951 gatctagttg ttcaaagcag cagaaaactt taaattttcc ttaattttga
2001 aagtgtgatt aatggaatat tgttacaatg ccattgattt atatactttg
2051 aggatagtta acttctttat gtttattaga aattgcactg agagctaata
2101 tgcagtttct attggtggtg atatgttctg ttaacagggc ctctgtcag
2151 tttttattct gagatttctg cctttctgtg tcttttgcat gagcctaact
2201 gactgagttt caatatcaag ttctgaaagc aagtgcagat actagtgtgc
2251 atgcaatgtg cacagaaaga tgtgttttcc tacctctcaa agctcaccaa
2301 aggatattac tcatattgcc acaaagtaca tttacaccaa taacacatac
2351 tttggtaatg ggaaaaataa ataattctag gtaataaaat gcactttggt
2401 gcttataaag gaaaataatc atcacaggta gaagaggagg aaggcaagac

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Exon 1

Exon 2

FIGURE 1B

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2451 taggttttga ttcaaaatgt tttctgtttt gctaggaagt ctagaaggaa
2501 tttggtcaaa tgacctgagg aggcaaggag atttctccca gtggcacatg
2551 gcagtcacaca agaagactag gggcagggaa acaaagcaag attgtatctg
2601 atttctacag gactccaaca ctaccctgcc aatacccttt tctcctctga
2651 aagccatgat cccaaagggt tccatttctt ccgggttttt cacattctac
2701 caacaagcag aatgtcttcc ccactgacag ctcaacagtt gagttttctg
2751 gggttctacc cttcatcaa cacagccttt gctttcctgg ttactgagta
2801 aataaaacac actggccact tctgcttagc cttcaggagc cactctggct
2851 ccaccctgaa gttccatttc ctccaagaac taagacattt ggagattctg
2901 cctaaaatat gcttaataaa aagggataaa ctgtctctca ctttctttcc
2951 cccacagctt gtcttaagaa caacggtgta accattagtg aaaaatcctt
3001 tcaggctagt aaaatgtaaa aagagaagag gtatcgggta acaacaaatg
3051 aaaataaaat cattcagtgg tagaaaccat aggagtgaat ttaggtagtt
3101 actgtcctcc agcttacatt aatcaaaata agcccacact gtcactaaga
3151 catcttgacg taagaagcca ttcaccctct cattgcaatt agtgtcataa
3201 agctgaatag acaggctcaga aatgaaaatc ataggccaat taagttattc
3251 tcatttttcc acctccagct agctcttttt gagaattttg attttaatag
3301 ataataaaac agtatgctaa ttgggtgggt ctgagcctga agccgtgaac
3351 tgaaggaaac ttcaacaaac atttggtctag ggctgctct aagcagaggg
3401 gctacgacaa tgggtaaggc atttctctag aaaccagcac tcagcagaat
3451 ttctggcacg agattttgtg agttttctag tgggtgtgtc tatcttcccc
3501 caatagatta taaactcctc gagggtgaca gaacacattg ctgatcttta
3551 tatectgcat gtttatggta ttcggtagac atttataaaa tgaattcatg
3601 aaaagcagaa catctagaaa tgaagtcaac attgcagaga agttagctat
3651 atgaaactct gtaaatcccc tcaaggaaga aggaagaagc agattttctg
3701 gaaatcaagg gacctcccag tataagagca attcctttgg tccaagaag
3751 ggcagctata agaacagaga cataactcct ccgtaagaaa agataaaaaat
3801 tctgaaagtt ctctaagaac atgccagacc ctgacctgcc tctcactctc
3851 agtagcatgg aagccgtact atctcttctc ttgacacaag catccacttc
3901 aatccacagg acaaatatca ccaatatgag aagataagat ctgttttata
3951 gtcaacttgg ctgacatgta ctaatttctc aaggctcagga tagctctcta
4001 aatagaaata cttgtttgtt ttaaggaatt ttatttaaat ttgtgtatct
4051 taaattttta ttttgatctc ggctcactgc aacctccacc tcccagggtc
4101 aagcaattct cctgcctcag cctcctgagt agctgggatt acaggcacct
4151 gtcaccatgc ctggccaatt tttgtatttt tagtagagac cggatttcac
4201 catgttaacc aggtctggtc caaactcctg acctcaagtg acctgcttac
4251 caaggctccc cagagtgtcg agattacggg catgaaccac tgctcccagc
4301 ctcatgtagt tcttaagagg aagaaaagcc tatagattag tgagaagtag
4351 acaaattagc aatttgaatc aaatgaaaac ttgggttgat ttcattcatt
4401 ttggagacac tttcgggtgt tccattttga tctgattcag gacactgatc
4451 ttcgattcta agttgcactg gttaatttgt gattatttta caaaatcata
4501 acagaatata tatctgggtc cagttcaagg tacagcaagc tactttcaat
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4601 acacacccaa atagaaacct caaaccaatt aagggtataaa taggtttagg
4651 atggggggagg atggacaaca aaaaaaacct cagtaaggct cctccacaaa
4701 gggcaccact tcagcttggg cccatgggca gacttcacct gtggcagggtg
4751 aaggggaaag cctaagacat cctgtgccct gatgcagatg tgacttacag
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4951 taactgggat gcatagggcc actccaggta ggcagcgagg ggtaaaccag
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5101 aaaccagccg tgatccttcc ttagtaacaa agtgtcaatc agtcatttgg
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5201 tgggataaat gaacattgtt atttaggtat gggttaaatga attacttttc
5251 aggagcagat ttatatatta acagggtgta aaaacttgaa tggataaatc
5301 atttataaag aaatttcagg aatcaactaa tcccaagggg aaagaccac
5351 taagacacta gattttgctg gagctactca aacaaattca tctatcatat
5401 ctacctaaagc cttccaatca atgagactag ccgcagtgac actgtctgtc
5451 aaaactacct cagtcatttt tttccaaggc aggaagcatc catacccctt
5501 cacaccttaa tctaatttcc cctatctcca cccactccc acttctatga
5551 ccccttccc cccatacca ccccggaagc atctactagc caacttagtg

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5601	aagttctgtc	acgctctcac	aaccacctct	gtgcagcaat	gattctgtaa
5651	atatccatgt	gtcctcaaca	ccagggtcaaa	ttagtccctt	tggtaaaaac
5701	attcattccc	tcaaattctc	ttccaatata	ctaataatac	ttcccaaaaa
5751	gtaaggagaa	gcttgaaagc	tagctggatt	gatgatggta	tgtgatgttc
5801	tataagttat	agttagtaag	catgttttta	ggatattttt	ctgctctcca
5851	aagagacaca	attccggaag	atatttactt	ttgtgtattt	ccacattttg
5901	gtttaagttt	ggagccatct	ctagatctta	atttcattcc	cctaatatgt
5951	gttatactag	tagaattttc	caaattacat	agaattataa	ctgcaactct
6001	tctgactgat	gccttttttt	tgcattttat	gatgcagttt	acatcacaaa
6051	ttctttccct	gcagggatta	tgtaaagagg	catgttgacc	tgctagccct
6101	atgttacttt	aagtatatgc	acacacacaa	aaaggaacaa	aaacagctgg
6151	gaattgatta	tgttgatatt	ctgaataaaa	gcaatagttc	taattatgta
6201	tgtctaatta	gccacagctc	ttcaagaatt	gctgcaaatg	tcacagggtt
6251	tataatgtcg	gcatttctatc	ttcacgaaaa	tgctatttga	tggcataaaa
6301	ccagaaaaaa	ctaattggtca	cagaagacag	cttgtagatt	agacaaaggt
6351	cactgtgttt	taatgaacag	tgctgttaat	taatgagaaa	acaactggta
6401	catgagcttt	taagcattgt	gaatttgtac	ccaaaaaatc	aatctgccta
6451	aaacaatttt	aagtagctaa	aaaacaaaat	aacggcaaga	acataattta
6501	aacctcaaat	ggtacagcag	agttatatgt	atcaattaaa	ttgaatcaca
6551	gttctcaggt	gtgacatatg	aagaggcttc	tttaatgcct	ggaaaagagg
6601	gttaatatgg	attgggtattc	tcaatacata	ttgtagataa	aattcaagac
6651	tagctctacc	actgcctctt	ttcttttttt	tttttttttt	ttatttgaga
6701	cagtatctcg	ctctgttgcc	caagctggag	tgcaagtggc	tgatctcggc
6751	tactgcaag	ctccgcctcc	cgggttcacg	ccattctcct	gcctcagcct
6801	cctgagtagc	tgggactaca	ggcgcccgcc	accacacca	gctaattttt
6851	tgtattttta	gtagagacgg	ggtttcacct	tattagccag	gatggtctcg
6901	atctcctgac	ctcgtgaccc	acccgcctcg	gcttcgaaa	gcgctgggat
6951	tacaggtgtg	aaccaccgcg	cccgccacc	actgcctctt	aggcttctta
7001	atttccttat	catttaagaa	gaataagaaa	atgcttctat	gttttaccaa
7051	aattctgtga	ggacaaatga	ggaaccattg	taactcctac	aaggtgagtg
7101	ataaaaaata	tacacattta	ttgtctttgc	ttttgtaaa	agttatccaa
7151	gccaaagctc	taggggctta	aataaggaag	gacaggacca	ttgttaataa
7201	catcaagttt	ccactacagc	tttctcccaa	acaagtcaaa	tattctgaat
7251	attattcact	aatctcttta	gctgccattt	cagtaaatag	cgagcatttt
7301	atttcaacta	aaaccaagca	agagaaaatg	aactgcttta	tcctgaggtg
7351	cagcagcaaa	ggcaccagaa	cttgtctcat	ggcttaccga	gcaagggtca
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7551	tggatttgaa	gaacacattt	cctcttacga	gtctatccca	ttgtctagat
7601	tgctggcaat	ggctttttta	aatttaaatt	gttattgaga	taattgtaga
7651	ttcacatgca	gttgtaagat	atagtagaca	tacctgtgtg	atactttacc
7701	caatttccgc	aaaaggtaac	attttgtaaa	actatagtat	aatatcacaa
7751	ccaggataat	aatattgata	cagctcacaa	<u>atctcattca</u>	<u>gatttctcca</u>
7801	<u>gttttacttg</u>	<u>aatacatttg</u>	<u>tgtgtgtgtg</u>	<u>tgtgtgtgtg</u>	<u>tgtgtgtgtg</u>
7851	<u>tgtgtattgg</u>	<u>ctctgtacag</u>	<u>ttttatcact</u>	<u>tgcagagatt</u>	<u>agtatatcta</u>
7901	<u>aaacaacaat</u>	<u>caagatattg</u>	<u>gacagttccg</u>	<u>gccgggcacg</u>	<u>gtggctcact</u>
7951	cctgtaatcc	cagcactttg	ggaggccgag	gcaggcagat	cacgaggtca
8001	ggagatcaag	accatcctgg	ctaacacggt	gaaaccacgt	ctctactaaa
8051	aatgcaaaaa	attagctggg	cgtggtgggtg	ggcgctgta	gtcccagcta
8101	ctcgggaggg	tgaggcaggg	gaatggcatt	aaccaggagg	gtggagcttg
8151	cagtgaagccg	agatcgcacc	actgcactcc	attctgggca	acagcgcgag
8201	actctgtctc	aaaaaaaaaa	aaaaaaaaaa	gatatacgga	agttccattg
8251	tcacaaggat	ctctcaagtt	accccttgct	aaccacatcc	accttctcct
8301	tcacacttgc	acaccccttc	ccttctccag	cctgacccca	gcagccacta
8351	atctgttctc	cattttctga	atgtttttat	ttcaaaaaatg	ttatataaat
8401	ggaataatac	agtgtataac	tttttaagac	tgacttttct	tgcactcaat
8451	ataattccct	ggcaattcat	ttatgttact	ctgtgtatca	atagttcatt
8501	catttttatt	attgagtagc	attccatggg	atggaggcac	cagagcttgt
8551	ttaaccatcc	tcatgggtga	ggacatctgg	gctgtttttg	gggtctggtc
8601	attatgaata	cttcttctgt	gaacattcat	gtacaggttt	ttgtacaaac
8651	ataagttttc	atgtctctga	cacgaatgcc	caagagtaca	attgctgagt
8701	catatggtaa	ctatatgtcc	agttttataa	gaaacgacca	tgctcagaag

D6S280

FIGURE 1D

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8751 gaccatactg ttttacatcc ccatcagcag tgtttgaatg atccagcttc
8801 tccgcaccc cccagcatt ttgtgtgtgc actatTTTTT gccactatTT
8851 tttatTTTT tagctctgct agctgtgtag tgataccatt gcgatctaatt
8901 ttgcattTTT ctgatggcta atgatgtcaa ataactTTTc atgtgtctct
8951 ttgccatatg tgtaacttct tttatgtatg tctctTTTTa tgaatatact
9001 atttatacct ttgccaatTT tctaattgag tttttggTTt tttactgttg
9051 agttttaagg tttctttaca tatttttagat attagtcctt tgtcagatat
9101 gtgggtttaca aacattTTTct cccagtcctat ggcttgtctt ttcacccTTa
9151 gtacctgggc tctcacagag taagtTTTTa cttttgatgg agtccaatTT
9201 ctcatTTTTt ctttttataa cttctgcttt tgatgtcaa agttaagaact
9251 ctttgcTTag tccaaatccc aaaaatatct ccattTTTTt cctaaaaagTT
9301 ttattatTTTT atgtttaatt tttaaacccg tgggtccattt ttaaagtatt
9351 atcataagat aggaagtata gattaaggTc cactTTTTtg cctatagatg
9401 tccaatTTTT ccagcatcat ttgttgaggg cccttcttcc tccattgaaa
9451 tgcttttgca cttttaaaac aaatcaattg agcatatttg tgtgggtcta
9501 gttctgaatt ctctattctg ttcactgttc tatatgtctg tatgttccat
9551 atgtctatcc ctccaccaat ccacagtctt gattactgga gctataatag
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Exon 3(D)

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FIGURE 1H

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23501 aaaccttcca gggcatcaaa gaagatagag gcaaaaaaaaa aaaaaaaaaa
23551 aaaaaaacta tccaaaggac agcaatttca aagactgaag gaaaatcagc
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24251 ttgtcagatt ctctaaggct aaaacaaaac aaaaaatgtt aacagcagct
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24351 acacctttca gcagaaactc tataagccag cagagattga ggacctatat
24401 tcagcattat taaagaaaag aaattccaaa caagaatttc atatccagcc
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FIGURE 11

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 24801 gctgtcttga atagacccat ttcacatgca gtgacgcaca taggatcaaa
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 25251 ggagtgtata tattcttctc atctgcacat ggcccataat ctaaaattga
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 26201 aacaacaaaa aacaaaactt caggccaaat ccttgatgaa catagatgca
 26251 aaaattctca ataaaaatcct agtgaaatga atccagcagc acatgaaaaa
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Phosphoglycerate
mutase,
processed
pseudogene

FIGURE 1J

27651	<u>gccccagggca</u>	<u>aagccaagaa</u>	<u>gtgaaggcca</u>	gcaaacaggc	accctccctg
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27751	tctatagaca	tcttgagttg	cagctgcaga	tggggaccgg	tggctcccat
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27851	gtcagaatag	cacttctagg	gcacagggtc	tcagtctaag	ctgtggaaaa
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36201   ttgctctgta   aatttaaacc   cataatgcca   ttgtttggct   gttgatgttt
36251   aaatattcaa   gctatgacct   tgaataagat   atccaagcat   ggaatttcat
36301   cataggatgt   tgggatttcc   agcagatgtt   tcaaatttga   ttattcattg
36351   gtggaatcca   aatctttttt   ttttaattct   gcagtcaaat   actggtatgg
36401   ggttctgttt   agaaatcaaa   actgactgca   gctttgtcat   ttggtatgtg
36451   tgaaaatagg   tactgaagaa   aattttaaac   atctgatggt   atcaacgtgc
36501   aaaattgctg   ttcttaaaag   ctgtctcaaa   ctttctatat   aaaagaatat
36551   aatataaagc   cactgtatca   ttgcctaaa   ccatgttcta   aagcatcaga
36601   aaatacacat   gacttttcat   ctcttcgatt   tattgaaaac   tgatggaatt
36651   acacctaggt   aatttactca   agatactaga   ctatgcctta   aataggatgt
36701   gcctctact   cattcatgat   gactctcagg   ggacaaatgc   ttctttctctg
36751   ttttataaat   acagaaacac   agcttcctta   cctccttcag   tctgtaaccc
36801   acatctgaaa   ttaaattgtt   gtgatcacac   tttgaagcaa   tttgtttcag
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36951   gccccattca   ccatctacca   aatgcctact   agccatctca   aaattaacat
37001   ggccataacg   gacaagtagt   tctgccttc   aaatagtgtc   cctcacctat
37051   cccagtggt   atttccccag   cttagttaa   agcactttat   tcacctgatt
37101   gcttagacca   aaaaccctga   agtcagcctt   agactccctc   gtttctctca

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FIGURE 1M

37151	tcccacagtc	agtccttcag	caagtcttgg	cctctccacc	ttcacaaatgt
37201	gcaccaaatc	cagctccttt	acagctcctc	ctgactttaa	cctcactctt
37251	gtaatgctgc	tctcaccctt	tgtccagacc	acagcagtg	cttcttacct
37301	ggccttccag	cttctctctc	gacatctggc	ttcctttctg	gcatccagcc
37351	tcttctcgtg	tggtttccca	aaggtaacca	aaatgatctt	gtaaaaataca
37401	catctgatcc	tgcactctgc	caaaaaccct	ttccttgccg	ctatcatata
37451	atacttagaa	ggacatctaa	agctattccc	aaagctgtcg	tgatctggct
37501	ggagccttct	ctcactccgc	tcaagctaac	ttgatctcct	tgccttcctt
37551	tgaacacact	gaacacactt	ccacctcaag	gtctttttat	atTTTTTtgc
37601	ctggaaccat	ttttccttag	atatgatcac	ggtcaccctt	tcacttcggt
37651	gggtcatggc	tcctctgaga	tgttttccca	ttgagacaac	cgaaaatact
37701	aaaaacggcc	gggtcagtg	gctcacgcct	ataatcccag	cactttggga
37751	ggccaagggtg	ggtggatcac	ttgaggtcag	gagttcgaga	ccagcctggc
37801	caacatggtg	aaacccccatc	tctcccaaaa	atacaaaaatt	agcctggagt
37851	ggtggtgcac	acctgtaate	ccagctactt	gggaagctga	ggcaggaaaa
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37951	gcactccagc	ctgggtgata	gagactgtct	caaaaaataaa	ataaaaaacta
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38101	ttcttggaac	ttcacattaa	gatagagcac	ccgcccctct	gtgactccac
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38201	tattctgtac	ttgtttattg	ctttaacatc	agcctacccc	agggaacat
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38551	tgagagataa	gtggtgacaa	agctagttaa	tggcgaacag	ataattaaac
38601	taggcatagt	tcaatgttct	ttatgcttcc	atgctttaca	tctctgtttc
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40201	tgatgttttag	aaacatttta	tctcacataa	ttcttctaag	acataatggt
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FIGURE 1P

46601 gaaaatgcc a g c a c a t a g t a a g t t t t a a g t g t a g t a t t t t c t g c a c c c c
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46751 t t a c a a t g a a a t a a a a g c a a a a t g t a t g t c c a a c a g g g t c a t a t a a g t a a
46801 t g t c t g t t t g t a a a c c a c t a g t c t c a g g a g t a a a t c c c t c a a c a t g a t g a
46851 a a a c c a a g a g a t g t g t t t g c a c a t a a c t c t t t t a t a a a g t t t t t t a a t
46901 t g c a a a g a c a t a g t c g t t g t t a t a a a c t t c c a a c a t a c a g a a t t a t a a a
46951 t t t a c a a a g t g a a a a g c c c c t t t a t g c a t a t a t t c a c t t c a g t c a c t c c
47001 c t t t a g a a a t a a t t a a t a g t a a t a a t c a g c g t a t a g t c t c a c t t t t t t t t
47051 t t t t t t t g a g a c g c a g t c t t g c t c t g t c g c t a g g c t g g a g t g c a g t g g c a
47101 c g a t c t t g g c t c a c t g c a a c c t c c g c c t c c t g g g t t c a a g g g a t t c a t
47151 g c c t c a g c c t t c c a a g t a g c t g g g a t t a c a g g c a c g t g c c a c c a c c c a
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47351 t t t c t a g t c a t a t a c t a a t a t a t t t t c t a t t a a a a t a a a a t c a c a t t g
47401 t a c a t a c t g t t t t g c a a c t g g c t t t a t t c a c t t a a a a t a t g t c t t t g g t
47451 g t c a g t g t c a a c a c a t t t a g t t c c t t c t t g t t t c c a a c a g c t g c c a a g a g
47501 t g g t a a c t c t c t t t g g c t c t c t g g g t a c t t a c c c a c a a a g c a c a t g c a t
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47751 c t t g g g a g g c t g a g g t g g g a c a c a g a g g t g c a g t g a g c a g a g c t a t g c
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47901 c a c t g c c a a c a a c t g a c c c c c c c t c t c t c a a a g c a c t g a c c c a a a a g
47951 g g g c a c c a g c a g a g a g g c t c a g a a g a t c t t c t c a g t c c a c a c c a g a g a c
48001 c a g a c c t t t c t c a g t g c a g g a g a a g g a t g a g a g a t g a a g c t g a a t g a g g a a t a c t c a
48051 c t t a t a c a g c a g t t a a t c c a g a g g a t g a c a g a a c a t t a c a a a t g t g a c a
48101 t c c t t g a a a t g g a a c t c c t c t t g g a g t g t g g a a g c c c a g t c a t g c a c a a
48151 c c c a c t t g g a g c a g g a a g g a a a t t g t a t t t g a g g a t g t t a a a g t c a a g c t
48201 a a c t t t t a t t a a a a t t g c c a c a g g a g g c c a t t c a t c c c c a c t t c a t t a t
48251 c c c a c t t c a a a g c c t a c t t c c a t c a c t c a g a t g t t a c t a t g g a g t g a
48301 t t a a g t t t t c t t t a t c t t t t c t a t g c t t t c a t c a g a t g c t c t t t t t c t c
48351 t t t t t t g c t t a g a a a a g g c a g a t t t t t t c a g c c t a a t g t c a a t a t g t c a a
48401 a c a t t a a g t a t t c a t t c c c t t g c a a t g c a c a g t g t t t a t a c t t a c a t a c c
48451 c t a t t a t a a c a a a t g t a a t a t t a t t t a a t t a t g c a g a a a c t t a c a c a t a
48501 a t t g g t g a a c a g g t c t a a a g a a t a c t c t a t a c a g g a a g c a t a g a t a t t c
48551 a g c t t a g c t g a g g c t t t g t t a a c a a t a t g c a a a g t t g c a g g t g c c a c a
48601 t t a g g g a a t t t t g g g g g a a a g g a a t g c a a c t t a t t t g g c a g g a a a c a
48651 a g a g a a a t g a t t a t a t c t c a c a g c a c t t t c a a g c t g g a c a c a t t t a c a t g
48701 c c t c t g t c a c a t c a c c c a g a a g c t g t g g t g t t g a g g g c t g a g g c a g g a g a
48751 t c c t t t t g c c a c a g g a a a a g g a t g a a a c g a g a g a c a t t g t g a a a g g t t g
48801 c c c c a a a g t c t t a a g g c t c c t g a a a c c t t g g g t t t t t g a a c a c c a t t g
48851 t c c a a t c t g t t g a a g g t t a a t a a t a c g t t g t t c a a a a t t g c a t a g g t a a
48901 a g c g c a g a a t g g c c a a a g g t c a g a a c t a c a g c a a t a g g t a g g t c c c a t t
48951 c t c t g c c t t t t c a t t c t t c a g t c t t c g g a a a t g c c a c a g a g g c c a a g t g g
49001 c a c t a g g t g c a g c t g g g a a t c a a g a t c t g t t a t c t g t a c c t t a a a g g t
49051 t c a a a g t g a a c c t t t t g g a a a t a t t g t t t t g g t a a a t a a a a a t t c c t g g t
49101 c g t g c t a a c t g t g g c t a t t t c g a c c t g t t t c t t c a g A T A C C A T T G T T C T
49151 ~~T A T C G C T T C A A T A G C A G T T G T T T C T G C A A A A A C T C A G G G T A A T A T T T T T G~~
49201 ~~C C A C G T C T G C A C T C A G A A G T C T C C G T T T C C T A C A G A T C C T C C G A T G G T G~~
49251 ~~C G C A T G G A C C G A A G G G G A G G C A C T T G G A A A T T A C T G G G T T C A G T G G T T T A~~
49301 ~~T G C T C A C A G C A A G g t a a g a t t g t c t c t g a a t t t a a a a c a c a a t t t t t~~
49351 g a a a c t t t t t c a t t g a t c g c t g t a a t a g t t a a t a a c c t g t c a c a g a a a
49401 a c t t a g t c a t t g c a g a a a a t g g t a a a a c a a t t t t g g g a c a t g a t g t a c a
49451 a t g g a t t a t t t a a g a g c c a t c a t a a a c a t c c a a g a g t a g t a a a g t g a a t
49501 a g a a t c c t c a t a g a a t t c t t t t t c c t t t t t c a a a g a t t t c c c a c c t c t a
49551 a a a a t c t t t c a t t c c t t t t a t a t c t a g G A A T T A A T C A C A G C T T G G T A C A T
49601 ~~A G G A T T T T T G G T T C T T A T T T T T C G T C T C T C C T T G T C T A T C T G G T G G A A A~~
49651 ~~A G G A T G C C A A T A A A G A G A T T T T C A C A T A T G C A G A T G C T C T C T G G T G G G G C~~
49701 A C A g t a a g t a t a a a a a t a c a t t t t t a t t t a t t g a t g t t g t g a a t t g t t

Exon 4(A)

Exon 5(B)

49751 tttttttaat acaacgtaat ggttcctatg gatggtttca ataaaaatat
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49851 taaaggcttt ccacatcttt ttaggccaac taagtgtgtc tgtaaacct
49901 cattaatatt tcttgagtgc tagattgttg ccaaaattaa aaatatataa
49951 aatatataaa tatatatcat ttttaaaatg gtgattaaaa cattgatagt
50001 ttgtttattt gttttaactt tgtggagcac aaatttaa at gatcaattgt
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52801 aagtgaata gatataaaag acctggtacc aagaacatag gaagtgccca
52851 atcaaatatg agagctattg ttggtgtaga catcattgct gtggaagtta

FIGURE 1R

52901 aatgaaagca cctttaatac aaaaggaaca aaaaaagagt tatgtgtttt
52951 gatgcagacc cacagtttga acacagaaga ggcttgggaa ttggagggtg
53001 tgatgaaaag atgtcctggg ttctgatgtg gcaactaaag ttgcattgat
53051 ggcagaaaat ggaagagggg agtagctcta gtagctgagg cactgagagc
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Exon 6(E)

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Exon 7(C)

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Exon 8

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Exon 9

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FIGURE 1AH

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Exon 10

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FIGURE 1AM

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Exon 11

Exon 12

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124401 CAGAGGCAGC CAAGATTTT ACCCCAAATG GAGGGAATCC AAATTGTTTA
124451 TAACTGATGA AGAGGTGGGT CCGGAAGAGA CAGAGACAGA CACTTGTGAT
124501 GCCGCACCGC AGCCTGCCAG GGAAGCTGCC TTGTCATCAG ACTCTCTAAG
124551 GACTGGAAGG TCACGATCAT CTCAGAGCAT TTGTAAGGCA GGAGAAAGTA
124601 CAGATGCCCT CAGCTTGCTT CATGTCAAAAC TGAAAATAAGT TCTTCATTTT
124651 CTTCCAGGC ATAGCAGTTC TTAGCCATA CATATCATG CATGAACAT
124701 TTCGAAAGCC CTCTTAAAAA GTTGAAATTG CAAGAATCGG GAAGAACATG
124751 AAAGGCAGTT TATAAGCCCG TTACCTTTTA ATTGCATGAA AATGCATGTT
124801 TAGGGATGGC TAAAATTCCA AGGTGCATCG ACATTAACCC ACTCATTTAG
124851 TAAGTACCT TGAGTTAAAA AGCCTGAGAA ACCAAACACA GCTAATGCTA
124901 TGGGGTGTAT GAATAGTCA AGTTAGGTC ATTTAGAAGA TTTGACACTG
124951 TATTTTGAAA TTATGGGAGT AAACACCTTC AAATTTGAGG CATTTCTGCT
125001 TTGTGACTAA ATACAAACTA CATTTTCAAG ATTAGGCCAT AATGTATATT
125051 TAAACACAAT GGCTATCAAC AGCTGCTAAT AAGGTATCAA CTAAAGCAGA
125101 ATTGGGGGAT AATAGAAAAT GCTGCTTATT TCAAGATATA TTTGCCAACCC
125151 CATTCCTATT CAGTCATTTT ATTATTAATG TAATTTGAAT GTCATTTTGT
125201 GTGGCTTTGG TGATTTAGCG CTGTGGCAAG CAATTTTGCA CATCATTTTC
125251 ATGTTGTTCT TTATGACAAG AATGTTCTTC AATTAGAAAA TGTGCAATA
125301 ATGAAATCA GGGCCAGTGA GGCAATAGA CTATCTGACA TATTGACTT

Exon 13

Exon 14

125351 TATGAAAACA TATTGCCTGA TGGCAGAATC AACTTTATAA GTGGTCAACT
125401 TCTACACAAG CGTATGAAAT ACTGGTCAGT AGAACAGCCA TTGTGATTGG
125451 ACTGGTTTCT CTGCAATGGC GCCAACCCCA GGCTTGCCAA TACTGCCTAT
125501 GTAAAGGGCA AGTGTGAGAA GCTATTCTCA TTTCGCTGAC ATACAGGTAG
125551 GACTATGGGG GATGGGACAT TTGAGTGGGA CTGAGATAGG AAAGGCTTGA
125601 AAAGAACCCA GAAACACCAC CAGGAAGTTG GCBAAGTAAA AGAAAAATGAC
125651 TTCCCCCTCA AAGGGCAATG AGAGGGAGAG AAACAAACCA AAATAGAAGA
125701 ACTAGACTTT TTAGAAAATG AGTATTGCTA GGGAAATCAA CTACCTAATC
125751 TTCCCTTATT CTTATATATA AGCAGAGAAT TTTTGCAAGG TATTTATTTT
125801 TTAATATGCC CTGAATGTCT TTTGCTATTA TGTGTACATT TTGCATATGA
125851 AAGTCTAAAA CGAAAGTTC TTTACTTTTT ATACTGTAGT GAAAATTTTC
125901 TATTCTTCCC AAGAAATGTTG TCCCBAATCT GAAATTACTG GTTCAATTTC
125951 CTGATATAAA

KCN6q cDNA

1 CTGGAGTGAGGCGCGGGAAGATGCCTGGTCTTGCCTCGCGGACTTGGCA 50
51 GCCGCGTCTGCGGGTCTGTCCACTGAACTGCTGAGGACTGCGGCGGTGG 100
101 CCTGAGGGAGAGCCGCCGGGGCAAGCAGGGGGCCCGGATGAGCCTGCTGG 150
1 M S L L G 5
151 GGAAGCCGCTCTCTTACACGAGTAGCCAGAGCTGCCGGCGCAACGTCAAG 200
6 K P L S Y T S S Q S C R R N V K 21
201 TACCGGCGGGTGCAGAACTACCTGTACAACGTGCTGGAGAGACCCCGCGG 250
22 Y R R V Q N Y L Y N V L E R P R G 38
251 CTGGGCGTTTCATCTACCACGCTTTCGTTTTTCTCCTTGTCTTTGGTTGCT 300
39 W A F I Y H A F V F L L V F G C L 55
301 TGATTTTGTGAGTGTCTTCTACCATCCCTGAGCACACAAAATTGGCCTCA 350
56 I L S V F S T I P E H T K L A S 71
351 AGTTGCCTCTTGATCCTGGAGTTCGTGATGATTGTCGTCTTTGGTTTGGG 400
72 S C L L I L E F V M I V V F G L E 88
401 GTTCATCATTCGAATCTGGTCTGCGGGTGTGTTGTGCGATATAGAGGAT 450
89 F I I R I W S A G C C C R Y R G W 105
451 GGCAAGGAAGACTGAGGTTTGCTCGAAAGCCCTTCTGTGTATAGATACC 500
106 Q G R L R F A R K P F C V I D T 121
501 ATTGTTCTTATCGCTTCAATAGCAGTTGTTTCTGCAAAAATCAGGGTAA 550
122 I V L I A S I A V V S A K T Q G N 138
551 TATTTTTGCCACGTCTGCACTCAGAAGTCTCCGTTTCTACAGATCCTCC 600
139 I F A T S A L R S L R F L Q I L R 155
601 GCATGGTGCGCATGGACCGAAGGGGAGGCACCTTGGAATTAATGAGTTCA 650
156 M V R M D R R G G T W K L L G S 171
651 GTGGTTTATGCTCACAGCAAGGAATTAATCACAGCTTGGTACATAGGATT 700
172 V V Y A H S K E L I T A W Y I G F 188
701 TTTGGTTCTTATTTTTTTCGTCTTCTCTGCTATCTGGTGGAAAAGGATG 750
189 L V L I F S S F L V Y L V E K D A 205
751 CCAATAAAGAGTTTTCTACATATGCAGATGCTCTCTGGTGGGGCACAATT 800

206 N K E F S T Y A D A L W W G T I 221

801 ACATTGACA[.]ACTATTGGCTAT[.]GGAGACAAA[.]ACTCCCCTAA[.]CTGGCTGGG[.] 850
222 T L T T I G Y G D K T P L T W L G 238

851 AAGATTGCTTTCTGCAGGCTTTGCACTCCTTGGCATTCTTTCTTTGCAC[.] 900
239 R L L S A G F A L L G I S F F A L 255

901 TTCCTGCCGGCATTCTTTGGCTCAGGTTTTGCATTAAAAGTACAAGAACAA[.] 950
256 P A G I L G S G F A L K V Q E Q 271

951 CACCGCCAGAAACACTTTGAGAAAAGAAGGAACCCAGCTGCCAACCTCAT[.] 1000
272 H R Q K H F E K R R N P A A N L I 288

1001 TCAGTGTGTTTGGCGTAGTTACGCAGCTGATGAGAAATCTGTTTCCATTG[.] 1050
289 Q C V W R S Y A A D E K S V S I A 305

1051 CAACCTGGAAGCCACACTTGAAGGCCTTGCACACCTGCAGCCCTACCAAG[.] 1100
306 T W K P H L K A L H T C S P T K 321

1101 AAAGAACAAGGGGAAGCATCAAGCAGTCAGAAGCTAAGTTTTAAGGAGCG[.] 1150
322 K E Q G E A S S S Q K L S F K E R 338

1151 AGTGCGCATGGCTAGCCCCAGGGGCCAGAGTATTAAGAGCCGACAAGCCT[.] 1200
339 V R M A S P R G Q S I K S R Q A S 355

1201 CAGTAGGTGACAGGAGGTCCCCAAGCACCGACATCACAGCCGAGGGCAGT[.] 1250
356 V G D R R S P S T D I T A E G S 371

1251 CCCACCAAAGTGCAGAAGAGCTGGAGCTTCAACGACCGAACCCGCTTCCG[.] 1300
372 P T K V Q K S W S F N D R T R F R 388

1301 GCCCTCGCTGCGCCTCAAAGTTCTCAGCCAAAACCAAGTGATAGATGCTG[.] 1350
389 P S L R L K S S Q P K P V I D A D 405

1351 ACACAGCCCTTGGCACTGATGATGTATATGATGAAAAAGGATGCCAGTGT[.] 1400
406 T A L G T D D V Y D E K G C Q C 421

1401 GATGTATCAGTGAAGACCTCACCCACCACTTAA[.]AACTGTCATTGAGC[.] 1450
422 D V S V E D L T P P L K T V I R A 438

1451 TATCAGAATTATGAAATTTTCATGTTGCAAAACGGAAGTTTAAGGAAACAT[.] 1500
439 I R I M K F H V A K R K F K E T L 455

1501 TACGTCCATATGATGTAAAAGATGTCATTGAACAATATTCTGCTGGTCAT[.] 1550
456 R P Y D V K D V I E Q Y S A G H 471

1551 CTGGACATGTTGTGTAGAAATTAAGCCTTCAAACACGTGTTGATCAAAT 1600
472 L D M L C R I K S L Q T R V D Q I 488

1601 TCTTGGAAAAGGGCAAATCACATCAGATAAGAAGAGCCGAGAGAAAATAA 1650
489 L G K G Q I T S D K K S R E K I T 505

1651 CAGCAGAACATGAGACCACAGACGATCTCAGTATGCTCGGTCTGGGTGGTC 1700
506 A E H E T T D D L S M L G R V V 521

1701 AAGGTTGAAAAACAGGTACAGTCCATAGAATCCAAGCTGGACTGCCTACT 1750
522 K V E K Q V Q S I E S K L D C L L 538

1751 AGACATCTATCAACAGGTCCTTCGGAAAGGCTCTGCCTCAGCCCTCGCTT 1800
539 D I Y Q Q V L R K G S A S A L A L 555

1801 TGGCTTCATTCCAGATCCCACCTTTTGAATGTGAACAGACATCTGACTAT 1850
556 A S F Q I P P F E C E Q T S D Y 571

1851 CAAAGCCCTGTGGATAGCAAAGATCTTTCGGGTTCCGCACAAAACAGTGG 1900
572 Q S P V D S K D L S G S A Q N S G 588

1901 CTGCTTATCCAGATCAACTAGTGCCAACATCTCGAGAGGCCTGCAGTTCA 1950
589 C L S R S T S A N I S R G L Q F I 605

1951 TTCTGACGCCAAATGAGTTCAGTGCCAGACTTTCTACGCGCTTAGCCCT 2000
606 L T P N E F S A Q T F Y A L S P 621

2001 ACTATGCACAGTCAAGCAACACAGGTGCCAATTAGTCAAAGCGATGGCTC 2050
622 T M H S Q A T Q V P I S Q S D G S 638

2051 AGCAGTGGCAGCCACCAACACCATTGCAAACCAAATAAATACGGCACCCA 2100
639 A V A A T N T I A N Q I N T A P K 655

2101 AGCCAGCAGCCCCAACAACCTTTACAGATCCCACCTCCTCTCCCAGCCATC 2150
656 P A A P T T L Q I P P P L P A I 671

2151 AAGCATCTGCCCAGGCCAGAACTCTGCACCCCTAACCTGCAGGCTTACA 2200
672 K H L P R P E T L H P N P A G L Q 688

2201 GGAAAGCATTCTTGACGTCACCACCTGCCTTGTTGCCTCCAAGGAAAATG 2250
689 E S I S D V T T C L V A S K E N V 705

2251 TTCAGGTTGCACAGTCAAATCTCACCAGGACCGTTCTATGAGGAAAAGC 2300
706 Q V A Q S N L T K D R S M R K S 721

2301 TTTGACATGGGAGGAGAACTCTGTTGTCTGTCTGTCCCATGGTGCCGAA 2350

722 F D M G G E T L L S V C P M V P K 738

2351 GGACTTGGGCAAATCTTTGTCTGTGCAAACCTGATCAGGTCGACCGAGG 2400
739 D L G K S L S V Q N L I R S T E E 755

2401 AACTGAATATACAACCTTTCAGGGAGTGAGTCAAGTGCCTCCAGAGGCAGC 2450
756 L N I Q L S G S E S S A S R G S 771

2451 CAAGATTTTTACCCCAAATGGAGGGAATCCAAATTTGTTTATAACTGATGA 2500
772 Q D F Y P K W R E S K L F I T D E 788

2501 AGAGGTGGGTCCCGAAGAGACAGAGACAGACACTTTTGATGCCGCACCGC 2550
789 E V G P E E T E T D T F D A A P Q 805

2551 AGCCTGCCAGGGAAGCTGCCTTTGCATCAGACTCTCTAAGGACTGGAAGG 2600
806 P A R E A A F A S D S L R T G R 821

2601 TCACGATCATCTCAGAGCATTGTGTAAGGCAGGAGAAAGTACAGATGCCCT 2650
822 S R S S Q S I C K A G E S T D A L 838

2651 CAGCTTGCCTCATGTCAAACCTGAAATAAGTTCTTCATTTCTTTCCAGGC 2700
839 S L P H V K L K 846

2701 ATAGCAGTTCTTTAGCCATACATATCATTGCATGAACTATTTGAAAGCC 2750

2751 CTTCTAAAAAGTTGAAATTGCAAGAATCGGGAAGAACATGAAAGGCAGTT 2800

2801 TATAAGCCCGTTACCTTTTAATTGCATGAAAATGCATGTTTAGGGATGGC 2850

2851 TAAAATTCCAAGGTGCATCGACATTAACCCACTCATTTAGTAATGTACCT 2900

2901 TGAGTTAAAAAGCCTGAGAAACCAAACACAGCTAATGCTATGGGGTGTAT 2950

2951 GAATATGTCAAGTTTAGGTCATTTAGAAGATTTGACACTGTATTTTGAAA 3000

3001 TTATGGGAGTAAACACCTTCAAATTTCAAGGCATTTCTGCTTTGTGACTAA 3050

3051 ATACAAACTACATTTTCAAGATTAGGCCATAATGTATATTTAAACACAAT 3100

3101 GGCTATCAACAGCTGCTAATAAGGTATCAACTAAAGCAGAATTGGGGAAT 3150

3151 AATAGAAATGGCTGCTTATTTCAAGATATATTTGCCAACCCTTCCTATT 3200

3201 CAGTCATTTTATTATTAATGTAATTTGAATGTCAATTTGTGTGCTTTTGG 3250

3251 TGATTTAGCGCTGTGGCAAGCAATTTTGCACATCATTTTCATGTTGTTCT 3300
3301 TTATGACAAGAATGTTCTTCAATTAGAAAATGTGCAAATAATGAAATTCA 3350
3351 GGGCCAGTGAGGCAAATAGACTATCTGACATATTTGACTTTATGAAAACA 3400
3401 TATTGCCTGATGGCAGAATCAACTTTATAAGTGGTCAACTTCTACACAAG 3450
3451 CGTATGAAATACTGGTCAGTAGAACAGCCATTGTGATTGGACTGGTTTCT 3500
3501 CTGCAATGGCGCCAACCCCAGGCTTGCCAATACTGCCTATGTAAAGGGCA 3550
3551 AGTGTGAGAAGCTATTCTCATTTCGCTGACATACAGGTAGGACTATGGGG 3600
3601 GATGGGACATTTGAGTGGGACTGAGATAGGAAAGGCTTGAAAAGAACCCA 3650
3651 GAAACACCACCAGGAAGTTGGCAAAGTAAAAGAAAATGACTTCCCCCTCA 3700
3701 AAGGGCAATGAGAGGGAG

FIGURE 3A

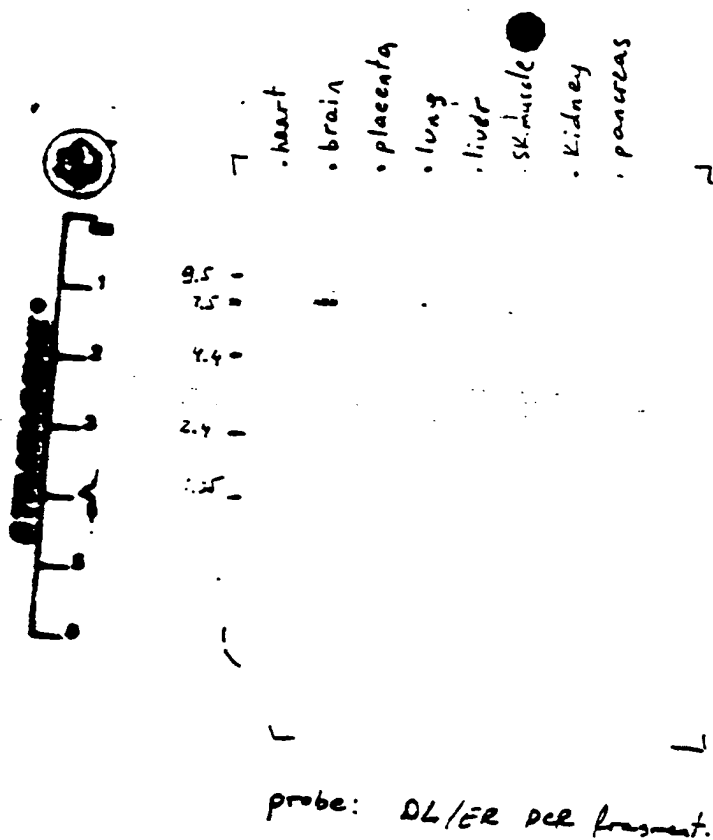


FIGURE 3B

RT-PCR analysis of the KCN6q gene expression in human tissues

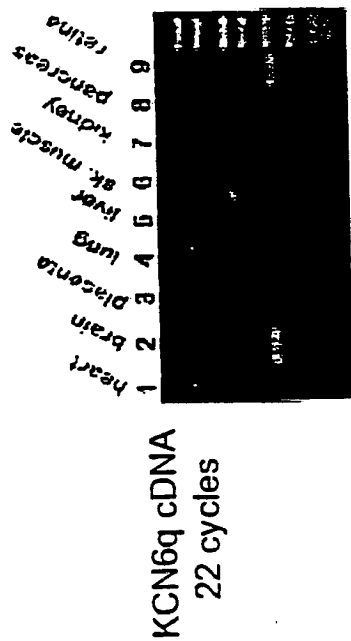


FIGURE 4A

KCN6q_MS.....LLGKPLS.....
 KCNQ4_ MAEAPPRRLGGPPPGDAPRAELVALTAVQSEOGGAGGGSPRRRLGLLGSPLPGAPLPG
 consensus maeappprrlgppppgdapraelvaltavqseqgeaggg sprrlgllg pl pgaplp

KCN6q_ YTS.SQS.CR.RN...VK.YRRYQNYLVNLERPRGWAFYVHAFVLLVFGCLILSVFST
 KCNQ4_ PGSGSGSACGQRSSAABKRYRRQNYLVNLERPRGWAFYVHAFVLLVFGCLILSVFST
 consensus Sgs Sac QR saa KRYRRVQNYLVNLERPRGWAFYVHAFVLLVFGCLILSV ST

KCN6q_ IPEHTKLASSCLLILEEVMIVVFGLEIRIWSAGCCCRYRGWQGRLEFARKPFCVIDTI
 KCNQ4_ IPEHTKLASSCLLILEEVMIVVFGLEIRIWSAGCCCRYRGWQGRLEFARKPFCVIDTI
 consensus I EH LA CLLILEEVMIVVFGLEIRIWSAGCCCRYRGWQGR RFARKPFCVID I

KCN6q_ VLASAVVSAKTQGNIFATSALRSREFLOILRMVRMDRRGGTWWKLLGSSVVAHASKELIT
 KCNQ4_ VLASAVVSAKTQGNIFATSALRSREFLOILRMVRMDRRGGTWWKLLGSSVVAHASKELIT
 consensus V IASIAVV A TQGNIFATSALRSREFLOILRMVRMDRRGGTWWKLLGSSVVAHASKELIT

KCN6q_ AWYIGFLVLIESSFLVYLVEKDANKESYADALWWGTITLTITIGYGDKTPLTWLGRILS
 KCNQ4_ AWYIGFLVLIESSFLVYLVEKDANKESYADALWWGTITLTITIGYGDKTPLTWLGRILS
 consensus AWYIGFLVLIF SFLVYL EKDN ePstYAD LWWGTITLTITIGYGDKTPL TWLGRIL

KCN6q_ AGFALLGISFFALPAGILGSGFALKVQEQHROKHFEKRRNPAANLIQCVWRSYAADERK.
 KCNQ4_ AGFALLGISFFALPAGILGSGFALKVQEQHROKHFEKRRNPAANLIQCVWRSYAADERK.
 consensus AGFALLGISFFALPAGILGSGFALKVQEQHROKHFEKRR PAANLIQ WR Y De SR

KCN6q_ .VSIATWKP...HUK...ALH.....TCS
 KCNQ4_ AYLTATWYVYDSILPSFRELALFEHVQARNGGLRPLEVRRAPVPDGCAPSRYPVATCH
 consensus a ATW yds L sfrelAL fehvqarngglrplevrrapvpdgcapsryppvatc

KCN6q_ .P..TKKEQGEASSSOKLSFKRVRMASP.R..GOSIKSRQASVGDRRSPSTHITRE.S
 KCNQ4_ RPKGSTSPCPGE..SSRMG.IKRRRMGSSORRTGPS.KQQLAPPTMPTSPSSQVGEATS
 consensus rpgst GEasss s Kervmas qRrtG sIK A SPstd aegts

KCN6q_ PTKVQKSWSFNDRTFRFSLRLKSSOPVIDADTALGDDVYDEKGCQCDVSVLDTETP
 KCNQ4_ PTKVQKSWSFNDRTFRFSLRLKSSOPVIDADTALGDDVYDEKGCQCDVSVLDTETP
 consensus PTKVQKSWSFNDRTFRFSLRLKSSOPVIDADTALGDDVYDEKGCQCDVSVLDTETP

KCN6q_ KKTIVIRIRIKFHVAKRKFKETLRPYDVKDVEIQYSAGHLDMLCRIKSLQTRVDQILGK
 KCNQ4_ KKTIVIRIRIKFHVAKRKFKETLRPYDVKDVEIQYSAGHLDMLCRIKSLQTRVDQILGK
 consensus IKTIVIRIRIKFHVAKRKFKETLRPYDVKDVEIQYSAGHLDMLCRIKSLQTRVDQILGK

KCN6q_ GOITSDEKSRK...ITAHETTDOISMGRVVKVEKQVQSIESKLDCLLDIYQOVLKRG
 KCNQ4_ G..PGDKAREKGDGSPSAEVVD...SMGRVVKVEKQVQSIESKLDCLLDIYQOVLKRG
 consensus Gqi DKK REKgdK e E DdlsMlGRVVKVEKQVQSIES KLD LL Y LR G

KCN6q_ .SASALALISFOIPPECEQTSQSPVDSKDISSGSAQ.NSGCISRSSTANISRGLOFIL
 KCNQ4_ TSAS...LGAVOMPLEPDTSDYHSPVDHEDRSVSAOTLS..ISRSVSTNED.....
 consensus tsASaLa QIP Fe e TSDY SPVD DLS SAQt SgclSRS S Ni rglqfil

KCN6q_ TPNEFSAQTFYALSPTMHSQATOVPISSQSDGSAVAATNTIANQINTAPKPAAPTTLQIPP
 KCNQ4_ TPNEFSAQTFYALSPTMHSQATOVPISSQSDGSAVAATNTIANQINTAPKPAAPTTLQIPP
 consensus tpnefsaqtfyalsptmhsqatqvpiqqsdgsavaatntianqintapkpaapttlqipp

KCN6q_ PLPAIKHLPRPETLHPNPAGLOESISDVTTCLVASKENVQVAOSNLTKDRSMRKSFDMG
 KCNQ4_ PLPAIKHLPRPETLHPNPAGLOESISDVTTCLVASKENVQVAOSNLTKDRSMRKSFDMG
 consensus plpaikhlprrpetlhpnpagloesisdvttclvaskenvqvaqsnltkdrsmrksfdmg

KCN6q_ ETLLSVCPMPKDLGKSLSVQNLIRSTEELNIQLSGSESSASRGSDQDFYPKWRESKLFIT
 KCNQ4_ ETLLSVCPMPKDLGKSLSVQNLIRSTEELNIQLSGSESSASRGSDQDFYPKWRESKLFIT
 consensus etllsvcpmpkdlgkslsvqnlirsteelniqlsgsessasrgsqdfypkwresklfit

KCN6q_ DEEVGPEETETDTFDAAPQAPAREAAAFASDSLRTGRSRSSOSICKAGESTDALSPLHVKLK
 KCNQ4_ DEEVGPEETETDTFDAAPQAPAREAAAFASDSLRTGRSRSSOSICKAGESTDALSPLHVKLK
 consensus daevgpeetetdtfdapqapareaaafasdslrtgrsrssosickagestdalsplhvkkl

KCN6q_ .MS.....LGGKPLS.....YTS.....SQS.CR.RN...VK.YRRVQN...
KCNQ4_ GGSRRRLGLLGSPLPFG...PLPG.....PGSG.....SGSACGQRSSAAHKRYRRLON...
KCNQ2_ AGS...EAPKRGSIILSKP...RAGG.....AGAG...R.....PPKRN...AF.YRKLON...
KCNQ3_ AG...ADKDGALLLEGGGRDEGORRTPEQIGILLAKTPLSRPVKRN...AK.YRRSOTLT...
KCNQ1_METRGS.....RLTGGQGRV
consensus ma k rr gaag gg pg gggaa pag al ag er vgl pgapd g alg

KCN6q_ .YVLERPRGWA.FYVHAFVLLVFCCLILSVFSTIPEHTKLA.SCLLILEFVMIVVFGLE
KCNQ4_ YVLERPRGWA.FYVHAFVLLVFCCLILSVLSTIQEHOELANECLLILEFVMIVVFGLE
KCNQ2_ YVLERPRGWA.FYVHAFVLLVFCCLILSVFSTIKEYEKSSEGALEYLLEIVTIVVFGLE
KCNQ3_ YDALERPRGWA.LYVHALVFLVLCCLILAVLTFKEYETVSGDWLLLELTFALTFEGAE
KCNQ1_ YNPLERPTGWKCEFYHFAVELLVLCILSVLSTIEQYAAALAVGLTFWMEIVVVFEGTE
consensus YvLERPRGwacfiYHafvLLvf CLILSVLSTIkeyeklasgcLLile vmivvFGL

KCN6q_ YIVRIWSAGCCCRYRGWGRLRFARKPFPCVIDTIVLIASAVSAKQGNFATSALRSL
KCNQ4_ YIVRIWSAGCCCRYRGWGRLRFARKPFPCVIDTIVLIASAVVIAAGQGNFATSALRSL
KCNQ2_ YFVRIWAAGCCCRYRGWGRGLRFARKPFPCVIDTIVLIASAVVIAAGQGNVFATSALRSL
KCNQ3_ YALRIWAAGCCCRYRGWGRGLRFARKPLCLIDIFVLIASPVVAVGNQGNVLATS.LRSL
KCNQ1_ YIVRIWSAGCRSYVGLWGRLRFARKPLSIDTIVLIASVVCVCAKGVFATSALRSL
consensus yivRIWsAGCccrYrGw GRlrfARKPfCvIdIiVLIASiAvVaaqtqGnvfATSALRsl

KCN6q_ RFLQILRMVRMDRRGGTGWKLLGSVVYAHSKELITAWYIGFLVLIFSSFLVYLVEKD....
KCNQ4_ RFLQILRMVRMDRRGGTGWKLLGSVVYAHSKELITAWYIGFLVLIFSSFLVYLAEKD....
KCNQ2_ RFLQILRMVRMDRRGGTGWKLLGSVVYAHSKELITAWYIGFLCLILASFLVYLAEK....
KCNQ3_ RFLQILRMVRMDRRGGTGWKLLGSAAKHAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEV
KCNQ1_ RFLQILRMVRMDRRGGTGWKLLGSVVYAHSKELITATTLTYIGFLGLIFSSFLVYLAEKDAVN.
consensus RFLQILRMVRMDRRGGTGWKLLGSvvYAhSkELiTawYIGFLvLlIfSsflVYLAEKD v

KCN6q_ANKEESTYADALWWGTTITLTTCIGYGDKTPLTWLGRLLSAGFALIGISFFALPAG
KCNQ4_ANSFSESYADALWWGTTITLTTCIGYGDKTPTWLGRLAAGFALIGISFFALPAG
KCNQ2_GE...NHEFETYADALWWGTTITLTTCIGYGDKVPOTWNGRLAAATFALIGISFFALPAG
KCNQ3_ DAOGGEEMKEEFETYADALWWGTTITLTTCIGYGDKTPTWEGRLHAATFALIGISFFALPAG
KCNQ1_ESERVEECYADALWWGTTITLTTCIGYGDKVPOTWNGTAAACESVFAISFFALPAG
consensus daqge andeFstYADaLWWGltItLTTCIGYGDktPtTWlGrllaa FtllgisFFALPAG

KCN6q_ ILGSGFALKVQEOHQHROKHFEKRRNPAANLIQCVRWSYAADEKS..VSIATWKP.....HL
KCNQ4_ ILGSGFALKVQEOHQHROKHFEKRRMPAANLIQAARWLVSTQ.MSRAYLTATWYVYQ...SIL
KCNQ2_ ILGSGFALKVQEOHQHROKHFEKRRNPAAGLIOAWRYATN.LSRIDLHSTWQYERTVYV
KCNQ3_ ILGSGFALKVQEOHQHROKHFEKRRKPAABLIOAARWYATN.PNRIDLVTATWYEVESVVSF
KCNQ1_ ILGSGFALKVQOQKROKHFNKQIPAAASLIOTAWRCYAAANPD....SSTWKIV.....
consensus ILGSGfALKVQeqhRQKHfEkrrnpAAnLIQaawRfYatd psr dl atWkye vtl

KCN6q_ K.....ALH.....TCS.P....TKKE
KCNQ4_ PSFRET....ALLPEHVORARNGGLRPLEVRRAPVPDGA PSRYPPVATCHKPGS..TSFC
KCNQ2_ PMGRLLHPLNQLLELRN.....LKSRSGLAFRKDP
KCNQ3_ PFERKE.....OLEAA.....S.....
KCNQ1_ ..IRKARSHTLSP.....S.....
consensus p frklp L qarngglrplevrrapvpdgapsryppvatcsrpg aftk

KCN6q_ QGEASSSOKESPKSRVRMASP.R..GOSIKSROASVGDRRSPSTDITAEQ.SP.KVKQSW
KCNQ4_ PGE..SSRMG.IKDRERMGSSORRTGPS.KQQLAPPTMPTSPSSQVGBATSP.KVKQSW
KCNQ2_ PPEPSPSQKVSILKDRVFS.P.RGVAAKGKGSPOAQTWRRSPSADQLED.SP.KVKPKSW
KCNQ3_SOKGLLDRVRISNP.RGSNTKGKFT.....PLNVDAIEE.SPSKEPKPV
KCNQ1_ .PKPKKSVVKKKKFKLDKDN..GVTEGEKMLTVB.....HICDPPEE..RRLDHFSV
consensus p apssSqliklkdrvrmsppqrgvp gKl tap tmrrspstd t Eatsptkv ksw

KCN6q_ SFNDRIRFRPSLRLLKSSQPKPVIDARTALG.TEDVYDEKGCQCVSVEDLTPLPKTVIRA
KCNQ4_ SFNDRIRFRASLRLLK..PR..TSAEDAP..SEVAPERSYQCHLEVDIMPVKTIVIRS
KCNQ2_ SFGDRIRARQAFRIKAAASR..ONSEASLEGEDVDKSCPCFVTEDTLPCLKVSIRA
KCNQ3_ GLNNKERFRKTAFRMKYAFW..OSSEDAGT.GUPMADRGYCNDEPIEDMPTLKAIRA
KCNQ1_ DGYDSVRKESPTLLEVSMPHFWRNSFAD..LDIEGETLLTPITHESQREHHRATFV
consensus sfndtrirfr rllkasap vqs eda pddvadek qcdsfvledltplk vir

FIGURE 4C

KCN6q_ IRIMFEVAKRKFKETLRPYDVKDVEIQYSAGHLDMLCRIKSLQTRVDQIVGQGQ.ITSD
 KCNQ4_ IRIMFELVAKRKFKETLRPYDVKDVEIQYSAGHLDMLCRIKSLQTRVDQIVGQG...PGD
 KCNQ2_ VCMIRFELVSKRKFKETLRPYDVMDVIEQYSAGHLDMLSRILSLQTRVDQIVGQGHAITDK
 KCNQ3_ VRIEQRFRYKFKETLRPYDVKDVEIQYSAGHLDMLSRILSLQTRVDQIVGQGHAITDK
 KCNQ1_ IIRMOXFVAKRKFKETLRPYDVKDVEIQYSAGHLDMLSRILSLQTRVDQIVGQGHAITDK
 consensus irimkflvaKrkPketlrPYDVkdVIEQYSAGHLDmlsRIKsLQTRvdQivgkqp it

KCN6q_ HKSREK.....ITAEHETTD.....DISMAGRVVKVEKQVQS...ESKLECLL
 KCNQ4_ HKAREKGDK.....GPSDAEVD.....DISMAGRVVKVEKQVQS...EHKLDLL
 KCNQ2_ D...RTKG.....PAEAEPP.....EDPSMMGRVVKVEKQVLS...EKKLDLL
 KCNQ3_ HKKSQKGSAAETFPSQOSPRNEPYVAR.PSTSEIEDQSMHGRVVKVEKQVLS...EKKLDLL
 KCNQ1_ ISVSEK.....SKDR.GSN.....TEGARLNREVDKVTQDQOHLALET
 consensus kk reKg ftfpsqqsprn p eaevp seiedismmgrvkvkVeqVqsiekkLd ll

KCN6q_ DRYOQVLRKG.SASATALLSPQPPFECEQTSQSPVDSK...DLGSAQ.NSGCISRS
 KCNQ4_ GFYSRCLRSQTSAS...LGAVQVPLEDPHITSQYHSPVDHE...DISVSAOTLS..ISRS
 KCNQ2_ NKYHORMG...IPPTETAY...FCYKEPEPAPPYHSPEDSHEH...DRHGCIIV...KIVRS
 KCNQ3_ DIRMHMER...LQVQVTEYPTKGTSSPAEAE...KKEDNE.YSDLK.....TICN
 KCNQ1_ DILHQLSLHGGSTPGSGGPPREGCAHITQPCGSGGSVDPELEHPSNTLFTYEQLTPR
 consensus diymqvlrkg sas lt appqigafapeq sdyhspvDsk yvdlgsaq s tirs

KCN6q_ TSANISRGLOFILTPEHPSAOTFYALSPTMHSQATQVPISSQSDGSAVAATNTIAMQINTA
 KCNQ4_ VSTNMD.....
 KCNQ2_ SSSTGQ...NFSAPPAA.....PEVQCPSST...SWQ.....PQSEHPRGHGTS
 KCNQ3_ YSETG...PPE.....PPYSFHOVTIDKVSPPYGFPAHDEPVNLPR.G.GPS
 KCNQ1_ GPDEGS.....
 consensus ts garglq ppe saqtifyalpp q t is a p n prag gts

KCN6q_ EKPAPTALQIPPLPAIKHPRPETLHPNPAQLOESISDVTTCLVASKENQVAQSNIT
 KCNQ4_ PV.GDEGGLVRIPPPAAH...ER...SDSAYGCGN.....RASMEPT..ROEDTP
 KCNQ2_ SCKVQATPSSATTYERPTVLPIITLLDS.....RVSCHS...QADLQ
 KCNQ3_
 KCNQ1_
 consensus p pa gtlq pp pa lerp tl ag esisdvttclras e vqv q dl

KCN6q_ KDRSMRKSPDMGGETLLSVCPMVPRDLGKSL.SVONLIRSTEELNIQLSGSESSASRGSO
 KCNQ4_
 KCNQ2_ G...CRPPECTLR...D...SDTSISPSVD...HEELERSPSGFSISOS...NL
 KCNQ3_ GPYSDRISPRQRRSITR...D...SDTFISLMSVN...HEELERSPSGFSISODRI..
 KCNQ1_
 consensus g s r s r tlrsvcpdvpsdt isl svqnlirsheelers sgfsisqsr

KCN6q_ DYPKWRESKLETTDEEVGPEETETDTFDAAPQ.....AREANFASDSRTGRSSS
 KCNQ4_
 KCNQ2_ DALNSCYAVAPCAKVRPYIAEGESDTSDLCTPCGPPERSATGEGPGDVGWAGPRK..
 KCNQ3_ DYPGPNQSSNREKR.YLAEGETDSTDPPFTPSGSMPLSSTGGG.ISDSVWTSPSNKPI
 KCNQ1_
 consensus dfl aa fi d r yiaagetdtdsd tp g p satgeg sdslwtg k

KCN6q_ OSICKAGESTDALSLPHVKLK
 KCNQ4_
 KCNQ2_
 KCNQ3_
 KCNQ1_
 consensus qsickagestdalslphvklk

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09587

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 1/00, 16/00; G01N 33/53

US CL : 530/350, 387.1; 435/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 387.1; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST DIALOG EMBASE MEDLINE BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KANANURA et al. The new voltage gated potassium channel KCNQ5 and neonatal convulsions. NeuroReport. June 2000 Vol. 11, No. 9, pages 2063-2067, see entire document.	1-10
Y	WANG et al. KCNQ2 and KCNQ3 Potassium Channel Subunits: Molecular Correlates of the M-Channel. Science. 04 December 1998, Vol. 282, pages 1890-1893, see entire document	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 JULY 2000

Date of mailing of the international search report

04 AUG 2000

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Form PCT/ISA/210 (second sheet) (July 1998)*

FIGURE 1A

KCN5g gene: DNA sequence

1. Underlined nucleotides in capitals represent exons.
2. Initiating ATG codon in exon 1 and terminating TAA codon in exon 14 are shown in bold italics
3. D6D280 genetic marker and phosphoglycerate mutase pseudogene are bold underlined
4. The exact lengths of the gaps between exons 1 and 2, 2 and 3, 10 and 11, 11 and 12, 12 and 13, 13 and 14 are unknown; these gaps are presented as runs of ten bold n as a convenience only

```

1  CTCGAETGAG GGCGGGGAG ATGCGTGGTC CTTGGCTGGC GGATTGGCA
5' GGCGGCTTCT GGCGCTCTGT CGACTGAACT GTGAGGACT GCCECGGTGG
101 CGTGAGGGGAG GGCGGCTGGG CGAAGCAGGG GGCGCGGATG AGCGTCTCTG
151 GGAGGCTTCT CTCTACACCG AGTAGCCAGA GCTGCCGCGG CACCGTCGAG
201 TACCGGCGGG TGCAGAACTA CGTGTACACG GTGCTGGAGA GACCTCGCGG
251 CTGGGCGGTC ATCTACACCG CTTCTCTTAA nnnnnnnttc ctttctatt
301 cttattatta atatatgac ttattattaa taatatgaag gaatagcaaa
351 tgagaatcca tgagcaatat cagccatga aaatgagcca gtggtgagt
401 aacaaccaat taggacactt gatagttag caaagttygc aaacaggaga
451 cagactcggc tcctttgaac gaagagtga tgcaagttyg attcceraga
501 taggagagca agaacatact ttctgggect ctctcaggat cgttcttgg
551 gaaggaaagt gtatgggaaa ttcccaaa cttagatgct aacattttaa
601 tgcaagcatg cacacacaca aacccacaaa caccccttt tttccatcaa
651 taaaattgca gaggagcccc atttgcacag tatatccat tgtattttaa
701 tatccaaat ggctagtccc ttccagagtt tttatgagtt aatgtgtgct
751 aatttaattg gcctggtgct ttattcattt gaagcaagaa attaagctcg
801 tgataataag gtaaggttct tatcagattt ctctttttgt tgctttacag
851 TTTCTCTCT GTCTTTGGT GCCTGATTTT CTCAGTCTTT TCTACCATTC
901 CTGAGCAGAC AAATTGGCC TCTAGTTGCC TCTTGATCTT Gttaaagttaa
951 acatgacaa gaactacat gaatgttga taagaactgc ctataacatt
1001 tatctatgc atcttatcct acaaaaaaat cctatctaaa aaagaggtac
1051 tgagaaatat aaaaatgtca aagattactg aaacatttgc ccaccaattt
1101 aacatgtagt caatccttag aaatatatag aaatgttcag gatttgcatt
1151 acacagcaat atcttgtgtt gtgatatat cataaataga aggaatatt
1201 agaaagcagt tttaagtatg tttctctatg ctaataaaca aattatataa
1251 gaagaaatcg tatctatgag gcctctcatt atattgtgaa agactataga
1301 gtgagagaca ttttccaaata actgtaattt ggcsgtagct aaatataatt
1351 ggccaagaac tatgaacata tggcacctca taagaaaata gaaggtctct
1401 tcagtctctt ttcaaccaac agactgcatt atgagttttg ctgctaagtc
1451 agttacctgg tgataaatte tgcagttttg tctgtttcca ttatgctgtc
1501 aatectcac cacacagact tgtcgaattc actttnnnnn nnnnnncgag
1551 gtcaggaggt cgagaccatc ctgctaatac cggtgaaacc cgccctctac
1601 taaaaataca aaaaattagc tgggcgtagc tactcaggag gctgagggag
1651 gagaatggcg tgaacctggg agggcgagct tgcagtgagc cgagattggc
1701 tcactgcact ccagcgacag agccagactc cgtctcaaaa aaaaaataaa
1751 aaaaaataaa gagtataact gatttatggc atgagtggtc ttgaatgatt
1801 ttgatggatg actggaaaca attagagata taaatcaata gcacagactc
1851 gtgacagatt tcattgaaga tacactgtga agcttcacat ggttaataac
1901 attgaattta ttaaaaataa agagactgca tataatagat ttttctttgt
1951 gatctagtgt ttcaaaagcag cagaaaactt taaatcttcc ttaatcttga
2001 aagtgtgatt aatgggaatat tgttacaatg ccattgattt atatactttg
2051 aggatagtta acttctttat gtttattaga aattgcactg agagcttaata
2101 tgcagtttct attggtgggt atatgtttct ttaccggggc ctcctgtcag
2151 tttttattct gagattttct ctttctctgt tcttttgcac gagcctaact
2201 gactgagttt caatatccag ttctgaaagc aagtgaagcat actagtgtgc
2251 atgcaatgtg cacagaaaga tgtgttttcc tacctctcaa agctccacca
2301 aggatattac tcattattgc acaaagtaca tttccacca taacacatac
2351 tttggttaatg ggaaaaataa ataatcttgc gtaataaaat gcactttggt
2401 gottataaag gaaaaataat atccacagga gaagagggag aaggcaagac

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Exon 1

Exon 2

FIGURE 1B

2451	taggttttga	ttcaaaatgt	tttctgtttt	gctaggaagt	ctagaaggaa
2501	tttggtcaaa	tgacctgagg	aggaaggag	atttctcccc	gtggcacatg
2551	gcagtcacaa	agaagactag	gggcaggga	acaaagcaag	attgtatctg
2601	attttacag	gactccaaac	ctaccctgcc	aatacccttt	tctctctga
2651	aagccatgat	cccaaagggt	tccatttctt	ccgggttttt	cacattctac
2701	caacaagcag	aatgtcttcc	ccactgacag	ctcaacagtt	gagtttctog
2751	gggtttctacc	ccttcaccaa	cacagccttt	gotttccctgg	ttactgagta
2801	aataaaacac	actggccaact	tctgcttagc	cttcaggagc	cactctggct
2851	ccaccctgaa	gttccatttc	ctccaagaa	taagaccttt	ggagattctg
2901	cctaaaatat	gottsaata	aagggatasa	ctgtctctca	ctttctttcc
2951	ccccacagctt	gtcttaagaa	caacggtgta	accattagtg	aaaaatcctt
3001	tcaggctagt	aaaatgtaaa	aagagaagag	gtatogggtg	aaacacatg
3051	aaatatccct	cattcagtg	tagaaacct	aggagtgaas	ttaggtagtt
3101	actgtctctc	agcttacatt	aatcaaaata	agctcacact	gtcactaaga
3151	cattcttgcag	taagaagcca	ttccacctct	cattgcaatt	agcttctata
3201	agctgaatag	acaggtcaga	aatgaaatac	ataggccaat	taagttatct
3251	tcatttttcc	acctccagct	agctcttttt	gagaattttg	attttaactg
3301	ataataaac	agtatgttaa	ttgggtgtgt	ctgagcctga	agcctgaaac
3351	tgaaagaaac	ttcaacaaac	atttggctag	ggcctgctct	aagcagagg
3401	gctacgacaa	tggttaagga	atttctctag	aaaccagcac	tcagcagaat
3451	ttctggcagc	agatttttgt	agttttctag	tggtgtgtct	tatcttcccc
3501	caatagatta	taaacctctc	gagggtgaca	gaacacattg	ctgtctttta
3551	tactctgcat	gtttatggta	ttcggtagac	atttataaaa	tgaaattcatg
3601	aaaagcagaa	cattctagaa	tgagtcacac	attgtcagga	agttagctat
3651	atgaaactct	gtasataccc	tcagggaaga	aggaagaagc	agattttctg
3701	gaaatcaagg	gacctcccag	tataagagca	attccttttg	tcenaagaag
3751	ggcagctata	agaacagaga	cataactcct	ccgtaagaaa	agataaaaaat
3801	tctgaaggtt	ctctaagAAC	atgcccagcc	ctgacctgcc	tctcactctc
3851	agtgacatgg	aagccgtact	atctcttata	ttgcacacag	cattccattc
3901	aatccacagg	acaaatata	ccaatatgag	agataagat	ctgttttata
3951	gtcaacttgg	ctgacatgta	ctaatttctc	aaggtcagga	tagctctctc
4001	aatagaata	cttgttttgt	ttasggaaat	ttatttaaat	ttgtgtatct
4051	taaaattttta	ttttgatctc	ggtctactgc	aacctccacc	teccagggttc
4101	aagcaattct	cctgctctcag	cctcttgagt	agctgggatt	acaggcacct
4151	gtcaccatgc	ctggccaatt	tttgtatttt	tagtagagac	oggatttcac
4201	cattgttaacc	aggttggtct	caaaactctg	acctcaagtg	atctgtttac
4251	caaggtctcc	cagagtgtcg	agattacggg	cattgaaccac	tgtctccagc
4301	ctcatgtagt	tcttaagagg	aaagaaagcc	tatagattag	tgagagtag
4351	acaaatttagc	aatttgaatc	aaatgaaaac	ttggtttgat	ttcatttcatt
4401	ttggagacac	tttcggtgtt	ttcaattttg	tctgattcag	gacactgac
4451	ttcgattcta	agttgcactg	gttaatttgt	gattatttta	caaaatcata
4501	acagaataca	tatctgggtc	cagttcaagg	tacagcaagc	cattttcaat
4551	gtttccagct	tggtgtttat	gattcataca	tgaatcattt	gggcattgcag
4601	acacacccaa	atagaaacct	caaaaccaat	aaggtataaa	taggttttagg
4651	atgggggagg	atggacaaca	aaaaaaacct	cagtaaggct	cctccacaaa
4701	gggcaccact	tcagcttggt	cccatgggca	gacttcacct	gtggcagggtg
4751	aggggggaaag	cctaaagacat	cctgtgccct	gatgcagatg	tgacttacag
4801	aacataaaac	gtaaaggcag	aagggatcat	ctgggtggct	gtctatctca
4851	ggcaggcatt	atagctgctg	acaagagcca	acccatrcct	aaggatctaa
4901	aatccctaca	agagaggacg	gaagtccctc	cattcatggca	ttccaaactag
4951	taactgggat	gcataggggc	actccaggta	ggcagcgagg	ggtaaacrag
5001	gaatgtctctg	ctgttccccc	ctcttttccc	caagcagggg	gcacccactt
5051	cctgctctca	gccccatagg	tctcaattta	tggtctraat	acctgatata
5101	aaaccagccg	tgatcccttc	ttagttaacaa	agtgtcaatc	agtcatttgg
5151	aatggggcat	tttcaaaata	ttgctccctc	atgatcttct	gaagaacctc
5201	tgggataaat	gaacattgtt	atttaggtat	ggttaaatga	attacttttc
5251	aggagcagat	ttatatatta	acagggttga	aaaacttgaa	tggataaatc
5301	atttataaag	aaatttcagg	aatcaactaa	ttccaaaggg	aaagaccac
5351	taagacacta	gattttgtctg	gagctactca	aaacaaattca	tctatcatac
5401	ctacctaagc	cttccaatca	atgagactag	ccgcagtgaac	actgtctgtr
5451	aaaactacct	cagtcatttt	tttccaaggg	aggaagcatc	catacccttt
5501	cacaccttaa	tctaatttcc	cctatctcca	ccccactccc	acttctatga
5551	cccccttccc	cccatatcca	ccccgaagc	atctactagc	caacttagtg

FIGURE 1C

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5601 aagttctgtc acgctctcac aaccacctct gtgcagcaat gattctgtaa
5651 ataccatgt gtccccaaca ccagggtcaaa ttagtccctt tggtaaaaaa
5701 attcattccc tcaaatcttc tcccaataca ctaatatacc tccccaaaaa
5751 gtaaggagaa gcttgaagag tagctggatt gatgatgga tggatgttc
5801 tataagttat agttagtaag catgttttta ggatatttt ctgctctcca
5851 aagagacaca attccggaag atatttactt ttgtgtattt cccatttttg
5901 gttaagttt ggagccatct ctagatctta atttcattcc cctaatatgt
5951 gttatactag tagaattttc caaattacat agaattataa ctgcaactct
6001 tctgactgat gccctttttt tgcattttat gatgcagttt acatcacaaa
6051 tcttttccct gcagggatta tgtaaagagg catgttgacc tgtagccct
6101 acgttacttt aagtatatgc accacacaca aaagggaaca aaacagctgg
6151 gaattgatta tgttgatatt ctgaataaaa gcaatagttc taattatgta
6201 tgtctaatta gccacagctc ttcaagaatt gctgcaatg tcacaggggt
6251 tataatgtcg gcatttcata ttccagaaa tgcctatttg tggcataaaa
6301 ccagaaaaaa ctaatggtaa cagagagacag cttgtagatt agacaaaggt
6351 cactgtgttt taaagaacag tgcgtttaat taatgagaaa acactgtgta
6401 catgagcttt taagcattgt gaatttgtac ccaaaaaatc aacttgccta
6451 aaacaatttt asgtacttaa aaaaacaaat aacggcaagg acataattta
6501 aacctcaaat ggtacagcag agttatatgt atcaattaaa ttgaatcaca
6551 gttctcaggt gtgacatatg aagaggettc ttaattgcct ggaaaagagg
6601 gttaatatgg attggtattc tcaatacata ttgtagataa aattcaagac
6651 tagctctacc actgcctctt tctttttttt tttttttttt ttatttgaga
6701 cagtatctcg ctctgttgcc caagctggag tgcagtggca tgaatcggc
6751 tcaatgcaag ctccgctccc cgggttcarg cctctctctt gctcagcct
6801 cctgagtagc tgggactaca ggcgcctggc accacaccca gctaattttt
6851 tgtattttta gttagagcgg ggtttcactt tattagccag gatggtctcg
6901 atctctgac ctctgtgccc acccgctcg gcttcgaa gcgctgggat
6951 tacaggtgtg aaccacggcg ccggccacc actgcctctt aggttctta
7001 atttcttat catthaagaa gaataagaaa atgcttctat gtttaccaa
7051 aattctgtga ggcacaaatg ggaaccttg taactctac aaggtgagt
7101 abaaaaataa tacacattta ttgtctttgc tttgttaag agttatccaa
7151 gccaagcttc tgggggctta aataaggag garaggacca ttgttaataa
7201 catcaagttt ccactacagc ttctctcaca accagtcaca tattctgaat
7251 attattcaat aatctcttta gttgccattt cagtaaatag cgagcatttt
7301 atttcaacta aaaccaagca agagaasatg aactgttta tcttgaggt
7351 cagcagcaaa ggcaccgaa cttctctcat ggttaccca gcaagggtca
7401 gaagaacct cctcaattta atcatctcg actgaatgta acagattttt
7451 gtattttcaa ctcctatgaa aataaaacaa tggagacctc tccaaaggtt
7501 gatttagaga gtacctctaa acaaaacaca gtgaaaatag acccagcate
7551 tggatttga gaacacattt cctcttccga gtctatccca ttgtctagat
7601 tgcctggcaat ggttttttta aatttaaat gtatttgaga taattgtaga
7651 ttccatgca gttgtaagat atagtagaca taacctgtgt atactttacc
7701 caatttccg aasggtaac attttgtaaa actatagtat aatatcaca
7751 ccaggaat aatattgata cagctcaca ctctcattca gatttttcaa
7801 gttttaetty atacatttg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg
7851 tgtgtatttg ctctgtttag ttttatcaat tgcagatatt acttatctta
7901 aaacaacat cagatatttg gacagttccg gacagttccg gacagttccg
7951 cctgtaatcc cagcattttg ggaggccag gacggcagat cagcaggtca
8001 ggagatcaag accatctctg ctacacaggt gaaccacgt ctctactaaa
8051 aatgcacaaa attagctgg cgtgggtgt ggcgcctgta gtccagcta
8101 ctcggggagg tgaggcagg gsatggcatt aaccaggag gtcggagctt
8151 cagttagccg agatgcacc actgcactcc attctgggca acagcgcag
8201 actctgtctc aaaaaaaa aaaaaaaa gatctcggac agttccattg
8251 tcacaaggat ctctcaagtt accccttget aaccacatcc aactctctt
8301 tcacaatttg acaccccttc cctctctcag cctgacccca gcagccacta
8351 atctgttctc cattctctga atgtttttat ttcaaaaatg ttctataaat
8401 ggaataatac agtgtataac tttttaagac tgacttttct tgcaactcaat
8451 ataattccct ggcaattcat ttatgttact ctgtgtatca atagttcatt
8501 catttttatt attgagtagc attccatggt atggaggcac cagagcttgt
8551 ttaaccatcc taatggtaga ggacatctgg gctgtttttg gggctctggc
8601 attatgaata ctctctctgt gaacctcat gtacaggttt ttgtacaaac
8651 ataaagtttc atgtctctga cctgaatgac caagagtaca attcctgagt
8701 catatggtaa ctatatgtcc agttttataa gaacagacca tgcctcagaag

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D6S280

FIGURE 1D

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6751 gaccataactg ttttacattc ccatcagcag tgtttgaatg atccagcctc
6801 tccgcattcct cccagcatt ttgtgttgte actatctttt gccattttt
6851 tttatttttag ccatctctgt agctgtgtag tgataccatt gccattcatt
6901 ttgcattttt ctgatggcta atgatgtcaa ataatcttct atgtgtctct
6951 ttgcattatg tgtacattct tttatgtatg tccctcttta tgactctctt
9001 atttatacct ttgcattttt totaattgag tttttggttt ttactgttg
9051 agtttttaagg tttctttaca tatttttagat attagtctct tgtagatat
9101 gtgggtttaca aacattttct cccagtctat ggcttctctt tcatcttta
9151 gtacctgggc totccagag taagttttta cttttgatgg agtccattt
9201 ctcatctttt ctttttataa cttctgtttt tgatgtcaag attaagaact
9251 ctttgcattg tccaaatccc aaaaatctct ccatcttttt cctaaaagtt
9301 ttattttttt atgttcaatt ttttaacccg tggctcattt ttaaatgatt
9351 atcataagat aggaagtata gattaaggto cacttttttg ccttagatg
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Exon 3(D)

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FIGURE 1G

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22401 gaatttcacc agtttttaca tgccttcaac ttttatatac actcctataa
22451 aattttctca catgtatata tgcatttaac caccaccaca ctcaaatcac
22501 aaaaactttt cctcacccta aataaacttg gaatgatata ctttgtatta
22551 ttcttttaca ggtactccct ctcttcaatc ctcaacctct ggaaccacta
22601 tatctgttct gtttcactgt aatttttgtc tttttataat gctatataaa
22651 tgaattgtta aagtagtaat ctittgaaat tggttttttc ttacacagca
22701 tgatgtcttt gagatccatc caagtgcate aatagtgcct tctgttttat
22751 tgcttaatag aattccatga tetagatgta acacattttg tatactcatt
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22851 aaggcagcta tgtacattca tgtgtagtgt tgtgtatgaa cgtaggtttt
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22951 aactatttcc ttttaaaaaa atgccaaca tgtgcctgga tgtgcctgga
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23451 attcttccag aactgaagcc agtcaactga accaccttcc tatgagatto
23501 aaaccttccc gggcatcaaa gaagatagag gcaaaaaaaa aaaaaaaa
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23651 agagtgtttt cttaacctca aacactgca gttagtccoc agcaatggtt
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23951 aatctcagag cttgaatata gcttctctga aataactcag tcaagacaaa
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24051 attatgtaaa gaccccaaat ctatgaactc ctgatgtccc tgaaagagag
24101 ggaagaaaaa caagcaactt ggaagacata ttccaggata tcatccatga
24151 aaattccccc aacttacta gagaggocaa cattcaaatl gaaaaatgc
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24251 ttgtcagatt ctctaaaggtc aaaaacaaac aaaaatgtt aacagcagct
24301 aaagagaagg ggcaggccac ctcccaggga ggctaacagc
24351 acacttttca gcagaaactc tetaagccag cagagattga ggccttat
24401 tcagcattat taaggaaaag aatttccaaa caagatttcc atatccagcc
24451 aaactaaget tcaaatga aggaataa agattctttt caggcagca

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FIGURE 1I

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24601	cgtgctlaag	tacacagacc	attgatgcca	aaaagcaacc	acacaaacag
24651	atctgcataa	taccacatta	acaaacacaa	acaggatttg	atccttcasa
24701	tccacacata	tcaatattaa	ccttaaacgt	aaatgggctc	aatgcccant
24751	taaaaggcac	agagtggcaa	gttgacacaa	gaagcaagac	ccaacagtat
24801	gctgtcttga	atagacccat	ttcacatgca	gtgacgcaca	taggatcaaa
24851	ctaaagggat	ggagaaaaat	ctactaagca	aatggaaaaa	agaaaaaagc
24901	aggagtgtgt	attctacatt	caaaccaaac	agacttttaa	ccaacaaaaa
24951	tcaaaaaaga	taaaagaaag	cattacataa	tggtaaaag	ttcacctcaa
25001	aaggccagac	ttactatccc	caaatagata	tgcattccaa	acaggagcac
25051	ccaaatccat	agagcaggtt	tttagacacc	cacaaagaga	tttagataac
25101	cacacacata	tagtgggaga	ccttaacatc	ccactggcag	cattagacag
25151	gtcattgagg	cagaaactca	acaaagaaat	tcaggacctg	aatttgacac
25201	ttgactaaat	agacctaata	gacatctaca	gaatctctca	cccaaaaaaa
25251	ggagtgtata	tattctcttc	atctgcacat	ggccacataa	ctaaaaattga
25301	ctaatcagcc	ataaaacaa	ccttagcaaa	taaaaaaa	tcataccaac
25351	cacactctca	gactacagtg	caataaaat	agaaattaat	ggaaattaac
25401	ctgcttctga	atgacttttg	ggtaaacaa	gaatttaagg	tagaaattca
25451	gaatttattt	gaacttaatt	agaaacaaag	tactacatac	cagaattctt
25501	gggacacagc	taaaacata	tttagagggc	agtttataga	gttgaattgc
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25601	agaggaatca	agaaacaaag	agcagatcga	ccccaaagct	agtagaacac
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25701	atcagagaga	tcaatgaatc	caggacctgg	ttcttcgaaa	gaatcaataa
25751	gtatgataaa	atgttagctc	gactaatag	gaacaaagga	gagatcdaaa
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25851	tttaaaaaaa	cctcagagac	tactaaaaac	acctctatgc	acacaaacta
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25951	attgaaccag	gaagaaatgg	aatcttgga	agagacaaat	aatgagttcc
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26051	agaggggatt	acagctgaat	attcaacaga	gtatnaagaa	gagctggtag
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26251	aaattctcra	ataaatccct	agtgaatga	atccagcagc	aatgaaaaa
26301	gtcaatccac	cactatccag	gaggcttcgt	cctggggaca	caagtgtgt
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26401	aaacaaaacc	acatgatcat	ctcaataaat	gcagaaaagg	cttttgataa
26451	agttcaacat	ccttccacgt	taaaaaccc	caacaaacta	ggcactgaag
26501	gaactactct	caaaataata	agagccatct	ataagaaact	caacagcaac
26551	atcacagctga	atgagcaaaa	gctggaaagca	ttcttattha	acacacagac
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26651	tcctggccag	agcaatcagg	caagagaaag	atataaangc	cacttgaaat
26701	gaggggaagt	caaatgttcc	ctgattccag	tcaatgatgt	tctatgccca
26751	gaacacccca	taattctctc	tcaaaagctc	cctggcttga	tcaaacactt
26801	cagcaaaagt	tcaggataca	aagtcaatgt	acaaacatca	gtagaaatcc
26851	tgtgaaocaa	caacatccaa	gctgagagcc	aaatcaagaa	tgttatcccc
26901	caagcctgcc	aaatgagcca	cctgaaacat	ggtgcaatc	cagcaaaagg
26951	agagtgaagt	gaacatgagc	aaacaaatca	aggtctgcta	ggacagacac
27001	ctgagtcacg	gggacatgga	ggcggaaag	aaagcactga	ggatgctgga
27051	ctatgagttt	gagctctggt	tcacatcaat	aaagaaagga	gagatccaaa
27101	ccttctggac	atggttgaat	gaaattgata	agatataagt	gaaatgagta
27151	aaagcttgag	gaaatgata	gaaacacat	ggggtctgag	ccgtctgaaa
27201	taaagcaaaa	atggttgaat	aaatagtgga	agccaaagtg	aaatatagga
27251	gtggtctcta	tgaatgacaa	caacatccga	tggagccata	caatctttta
27301	taaagcaaaa	tcaatgagga	tcaacgttat	gaaacacata	gaaacaaata
27351	gctaaagcca	tctgagagtc	tgaaggaagc	tattgacaga	gaaatgacat
27401	tctgagagga	agaaatattt	caaaagacaa	agaaaggaag	atggttaagt
27451	aatgcaagcc	atggtgagga	cctatgggga	aatgcaagga	atctgaaagg
27501	tctatctgaa	gaggtctcta	tggagctgaa	aatgcaagga	gaggtctcta
27551	ttgtatctga	attggaacaa	aaatgaaagc	caatcaagga	aaagctctga
27601	agttcaagga	ggtggaagga	aaatgaaagc	aaatgaaagc	aaatgaaagc

Phosphoglycerate
mutase,
processed
pseudogene

FIGURE 1J

27651	<u>ggncaagggca</u>	<u>aaacaaagaa</u>	<u>gtgaagggca</u>	gcaaaacagge	acccaccccg
27701	cccattgcat	ccatctgtcc	ctccctctcg	aacatgtcac	actgaccaca
27751	tctatagaca	tcttgagttg	cagctgcaga	tggggaccgg	tgggtcccat
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27851	gtcagaatag	cacttctcgg	gcacaggttc	tcagtctaa	ctgtggaaaa
27901	gcccccggtt	tccaagagag	ttcaaaagata	gtgacttggg	tttttgcag
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28301	acatgtggtc	atcagaaeat	aaagccattcc	tcataccaat	atggggtlaa
28351	ctccttgacc	tttgaggggc	aggagtgttt	catgtctgtg	gttttagaat
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28451	gtgtgttttt	cactgatttc	tgaatcatgt	tgcagttgct	tggcctctgc
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28601	atgcacacca	gttagaatgg	tgaacattaa	aaagtacagg	aaaaacaggt
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28701	tgtaaacctag	ttcaaccatt	gtggaaagaca	gtgtggtgat	tctttaaggga
28751	tctagaacta	gaastaccat	ttgaaccagc	catcccatte	ctgggtatat
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28851	tttattgcag	cactattcac	aatagcaaa	acttagaacc	aaacaaaag
28901	tccatccatg	atagactgga	ttcagaaat	gtggcagta	tacccatgg
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30701	atggctatta	ctaaaaagtc	aaaaaatcac	agatgctggc	aagggttatgg
30751	agaaaaagga	atgcttatat	actgctgggtg	ggaatgtaaa	ttagttcagc
30801	cgggtgtggaa	agccgtttgg	caatttctca	aagaaactca	aatpgaatca

FIGURE 1K

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31051 aaaaagacca agatcatgtc ttttgcaaca acatggatga ggctggaggc
31101 cattcaactc agcgaactga cccaggaaac gaaaaccaaa tacatctgtt
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31351 acctgcacat gtaccacaga aactgaaata aaagtttaaa aataaatcat
31401 aaaataaaat gttgaaatgc ctagaatgtt tcatagcatt aaattagacc
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32601 ctcaactata aaaaagaaat gcaaatgaaa gcattctctc acctatcaga
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33851 gaggtcaatg gaaggagcaa gaaccagac aggtactttt tgagtctggg
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34201   agccatgccc   tttaatatta   tatgccccagg   tcttagaagt   ttctggccac
34251   aatgaatgtg   tattgaatga   ataanaaatt   gagaaaactg   caagctagta
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34351   acacacaaag   taanaacaa   acagtcaaca   tgagcaata   gagaaaaaaa
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34451   agttatcaga   ggttttcaaa   aggtatattt   ttgctgtcat   tttttaatct
34501   aattgcttca   attattttat   ttggtcatgg   gaacatgatt   atatttttta
34551   ataaagtgtc   taatttgcct   gcctgctttg   aattgtaggt   tcttttacag
34601   gagcctgtc   tactcccata   ttgctttttt   ctcaattatt   ttacttctgc
34651   cttttgcaaa   atgttttaaa   ttttgyattt   tagccctac   cacagactcc
34701   aaatctgtgc   ttggaatttg   gagatcaaa   tactgtagct   aatccttagg
34751   acatttgcca   ttgtgtgact   taagaaacca   tctcttatca   agttctctgt
34801   gttttctgga   gtatttacag   gtcacaatgt   caaatgtgtc   acatctgaaa
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34901   aaaaatatca   gctcctgaat   ggtattgaac   togattagcc   catctccagt
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35051   ecagtaggaa   aggaattgat   aatgaaaaaa   aagtgtgtcc   gaaggaggtt
35101   ttttctctcat   agcttttagg   ttgtttataa   gacaatgtgt   ttccccgctt
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35301   acagcaatcc   ataaaagtaa   atatgaattt   agaaagtccc   aaaaactgct
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36651   acacttaggt   aatttactca   agatactaga   ctatgcctta   aataggatgt
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36901   aaaaaatcta   ctgctgact   ctgcatctct   gaactttccc   ccagactcca
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37001   ggccataacc   gacaaagtgt   tctgccttc   aatagtgct   cctcacctat
37051   cccagctggt   atttcccag   cttagtaaac   agccctttat   tcaactgatt
37101   gcttagacca   aaaaccccta   agtcagctt   agaactccct   gtttgtctca

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FIGURE 1M

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37251 gtaatgctgc tctcaccctt tgtccagacc acagcagtgq cttcttacct
37301 ggccctccag cttccctctt gacatctggc ttcctttctg gcctccagcc
37351 tcttccctgt tgggttccca aaggtaacca aaatgatctt gtaaaataca
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37451 atacttagaa gacatcttaa agctattccc aaagctgtcg tgccttggct
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37751 ggccaagggt ggtggatcac ttgaggtcag gaggctcgag ccagccctggc
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FIGURE 1P

46601	gaaaatgcca	agcacatagt	aagttttaag	tgtagtattt	tctgcacccc
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49701	<u>ACGgttaacta</u>	<u>taaaaataca</u>	<u>ttttttattt</u>	<u>atggatgttt</u>	<u>gtgaattggt</u>

Exon 4(A)

Exon 5(B)

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FIGURE 1U

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Exon 6(E)

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Exon 7(C)

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87101  ttgaaaataa agcaaaaaag atagtctcag tgaagtgtcc atccaggcat
87151  tccaatatcc ttatcaaaag aatcaacat ctcaagtga gggaaactaa
87201  gaggaagatt ggcacagta gaagagctca tatgattctc cagacagga
87251  ggacaggagt gcacaccaag attatcacag ccaagtgtcc agcactgtga
87301  accaaaaact tcttaaatgc tttctgagca tanaatttca aaaggaacaa
87351  caaaggggga gacataggat ttctcattag gacagagcc aaagaagac
87401  gaataaggat tactttcgaa attatagaa ttattttcaa ccttagaacg
87451  aatgaacaa tatatccagc caaacacca tcaattctga gacagaccc aaggcatttt

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87551 taggcacgca agatctcaca aattaatcra ccattttacot ttactcaaga
87601 aactttgtaa ataggaacttc tottccacaa caaggggata agccaagaga
87651 gaagagagaca tggagtgcaag saaacaaagag attccgcaca ggaagagaca
87701 aaggggatttt ctatgataat aggyaaggga agtcccaaga caacgcctat
87751 gcaggcctttg agaacagaaat gagaaggagg ctgagagcto caggagaaag
87801 atcttcaagg aagcgaaaga atggagttct cagatttcat gatgcatttg
87851 accagaggaa ttttatagtt ctgttgpaaa gatctggcat gtaacataa
87901 caggtacata taggcaaatc aagaatttaa aatagggcaa ttataactcc
87951 aaaaaaaccc aaaaacttga taagaagaga agagcagcca tagtcatca
88001 cattgctgaa ctgggaacag cagttacatc gtcataataa tattaatag
88051 taacttagot aagaacttgy atatttaaat gttgatagga tgaaggagg
88101 ggaagagggc atgttaagtgg gcaatatcat tatcgttccg gataggaggt
88151 taattgataa tgtctaaast tgaataatca agaatataa ctataagcat
88201 aatcctkaaa aataagcatt ctgtgttggg cgaagtggc tcatgctgt
88251 aaccccccga ctttgagagg ctagggtggg aggalcactt gaggccagga
88301 gttcaagacc aacctgtgca acatagcag agccacctc tacaazaaa
88351 ttttaaaaaa tcaagtgcac tggcacacac ctgtgtctct cacacacctg
88401 tagctactca ggaggtgaa gccagaggat tgggtgagcc tgggagtttg
88451 aagctgctgt gagctataat tctaccactg cactccagcc tgggtgatag
88501 agtatgaccc tgtctcaaaa ataataataa taataataa ggattottaa
88551 aaatagaagc acaactata agaattgaag gggttgcttc tgaggaaacg
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88651 aactttcagg aactttcacat tttaaacctt gtacottgaa asaataagct
88701 ctgtgttcat tattcctttg gatgtctagc tgaacgttc taaccattt
88751 ccagtaagga tcaaacatgc cataatggtc atacttgcac gacaaacaaa
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88901 aagttggcto tcatitgtct ccttgttgc ttgtcctttg aactgtatac
88951 accatgtaaa ttaacctag gaaatccctc taagtgttat tttctcttg
89001 ccaactactg tgtgattttg gtttttaata taggaacatc agctactgga
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89251 gagtaagtaa ggaacaaaga gcttagctgt tgggpatcaat ctgagagaaa
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89401 ggtcccatct tttgttgtt ctgttcttt tatgtctcta gttctacag
89451 aataatgtgc agactgcaa tatgtgtgac atcttctatg yaactgttt
89501 tcaatgtatc aaaaatcata tctgtctgac tctctcaaac attagaggat ctctgcat
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89601 cactactata actctataaa ttgtgcaggt ataatatttg caaagattaa
89651 attctaaatc catttcaaga tgatgcacaa atagttagcat tataabagat gtaagatac
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90251 tttctgggta ttttggaaat tccagtttta gtttattaga aaccaacata
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90501 acattttctt cattatggat ttcacaatca tgaactgtgt gttatgtact
90551 agggatgtgg cccattttaa cgaattggct tggcaggcat ggtggcagat
90601 gcctgtaatc ccagcatttt gggaggccga ggtggcagat cccctgaggt
90651 gaggagttca agatcagcct ggccaacata gtgaacctt gtctctacta

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90801   gcagacagcc   aagattgtgc   cactgcactc   cagcctgggg   gacagagggga
90851   gacccgtgtc   caaataaata   taataataat   aataagytgt   gtacacataaa
90901   ataaaatgtt   ttgattaate   taataataat   aaaaagtgtt   ctctgagggg
90951   actggagtaa   atacctggcc   attagagatg   ttgacagact   cttaaaaaag
91001   aaagaaattt   acaaaaatct   atatgggaaa   gtaaggtegg   gcaatctctc
91051   aaatgagcag   ttcagatgga   agctttttcc   ataacagtct   tcaagcagtc
91101   agcaagtctc   ctgtgagggg   ttctatgact   gacagccagg   caatctgaga
91151   tattgtctca   actccacca   ctgtgtataa   accctcttcat   ttatctgagc
91201   cctttttttc   tcaacctaca   aaagggaatc   ttaattcaca   cttgtatggt
91251   gaggatcaca   taaaataact   ttgtcttatg   tatgccaggt   gtaattatct
91301   gaaatggaga   gatttgatgt   ttacatcaa   agagctataa   acatattctt
91351   agatctggc   ataatccgt   aggtgttttg   cagctataca   cctagagagt
91401   acattttccc   catcttgttt   tagttctgt   totgtatctc   actatcttca
91451   tttttagcat   tagaagtatt   acctagtcca   aggatttcat   taattcaatc
91501   actagcatac   cctgggtgtc   attagttaac   tagttagctt   actagtgtca
91551   taatggattt   tcaattagtc   ttgtcttctc   ccttatcmaa   tattgattaa
91601   agatcaatct   ctagaatgta   tgcctcatgt   caagtgcctc   acctaaactac
91651   tatcttggat   attagcattt   aaccagtcaa   tcatcacttt   ttgttttggg
91701   taatatagta   tctttccctt   accaattcaa   aaatcacatt   atattgcaat
91751   tttttgggct   tttgtttctt   gctatgttgt   tttgaaagga   agctctggcc
91801   aggcacagt   gtcataact   gtaatccag   cactttggga   ggcacaaggca
91851   ggcggaccac   ttgaggtcag   gaatttgaga   ccagcctggc   cagcatgggtg
91901   aaactccctc   tctactaaa   gtacaaaaa   aaaaaaaat   agccaggcat
91951   ggtgtgtggc   acctgtagtc   ccagctactc   tagaagctga   ggcacaaaga
92001   tcaattgaac   ccagaaggca   gagattgcaa   tgagctgaga   tgcaccact
92051   gcaactccgc   ctgggcaca   gattgagagt   cctctcaca   ataatataat
92101   aataaaatca   aataaaata   aagaagtctt   gatgtgtgtc   aatgtgttta
92151   acagccagct   gaaaatggca   aagtagcttt   ctctctctct   tgggtattta
92201   tcagggctaa   gtataagagg   caaagatttc   ttcatgtgta   gtaacttaac
92251   catctctctt   tttattttat   tctaaatcct   gctctctttt   ttcttttca
92301   tgtcccccac   tatgcacat   gcagtggttt   tggcctagtt   acccagctga
92351   TGAGAAATCT   GTTTCATTG   CAACCTGGAA   GCCCACTTC   AAGGCCCTGC
92401   ACACCTGGAG   CCTACCAAG   taggtatcag   tgtgacagct   gccactgtag
92451   ttgagtcttt   tcaagcttgt   taacaaacc   ataccacac   ccacaaaaaa
92501   aggtgttaat   gtttggcttc   tcttattctc   tgttgctctc   agagagattc
92551   aaacttattt   agtagcagtt   ctggcaccac   actaaatgga   atcagtatac
92601   tctctacaa   tccacagact   ggggttaag   attaaatcaa   tgtaaatttt
92651   atagagattt   atttttacta   ttctatgctt   accttctaat   gtctcatttc
92701   gtaaaagcca   ccagcatcct   aaaggttaag   accacagttc   ttgaaaaaca
92751   aggatttttt   attttgccc   catgccttga   ccccaagaga   tgagtgttta
92801   gagagtaac   catctttgta   cgtttagatc   totcaagcc   attctctate
92851   tggatctctc   ctggtatggt   tccaagcttt   tacttgca   tcasactcac
92901   atcagtggca   aaaaaagaga   aaaaatagg   tctaatagg   atttatgttt
92951   acaatgaggg   aatgggacc   agcggattaa   aatttggaa   ttgcattcac
93001   aatttttaag   taagactact   tttcttcaca   tttcattggc   caaagttaat
93051   cactcagcca   tgcttaatt   cwaagaaat   ggggaagtac   aattttacct
93101   tttaccatgt   gcttagagc   aggcagaac   caaggacatt   atggacagtc
93151   ctatgatta   ccacagagat   ctgtaattaa   aaaaaaaac   aagggtctag
93201   tcttgagatc   ttggaaacct   ggcctccagt   tcaactatca   cagaaaaagt
93251   taatcatat   tcaaacctta   attaaagaag   tctctatgga   ataccagaag
93301   cagtaagtc   tgagaaaggc   tttctgtggg   tcaaccagct   tgattagcgt
93351   agtcagttcc   tdaggttgtt   caaaagttaa   taattgtcc   atcgacatt
93401   tgagagcacc   agctgttttt   ctctatagcc   caacaaaacc   ttaactctag
93451   tggcttcatg   ggattgggga   caactaacta   ctgtgactca   caaaaaacca
93501   cctgttctga   ctgatcttgg   gatatacag   ctgaggaaac   ctccatgaca
93551   ttccctggca   gtaccgtctt   aatggaggtt   tgtgatgcag   cagaatgcct
93601   accattggga   agcaccagat   ccatcattaa   ctacattact   tccctgttct
93651   gcccctggcc   ttagtttttc   caaccacaaa   gagaaggaca   agagtgggac
93701   aagatgattc   ctaagctcta   atgggaaatg   cctctctgtg   ggtgttatct
93751   aaattcttg   tcagcaccat   ggacagagat   attcatagca   ctccagggat
93801   ttcagagagg   atggaacctc   agcatggata   gaagcacgga   tgcgttgaa

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Exon 8

FIGURE 1AE

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93851  tgcattgtttg gattaaaato cttttttcat ttagatcttg aagtttagct
93901  gaacttttta tggagtttaa gaccttttca acattttgat atc333333ac
93951  cga3333taga acctaaactcc tgtgccatit atctcttggg agtatgaacc
94001  a333333333 attatataga aattgtatit cttgtcttca tgaagtttgc
94051  taggtaaatg taaaaggga atctgagtag gaaactgat agagaattct
94101  caagtgcacg aggagagtgg tctattatca tatgtgatta tttggcttcc
94151  caattactgc agtttttggg ttaaaagtca ttaaaagcct gg333333actg
94201  ttt333333atg agtcaaatte cccagtaaa tcaactatta ttttatccac
94251  ataagttctt tctactcttc tgatatitct cctgtcttaa gtgggggttg
94301  aggtggagga ggatttatgc aaacacctgt gtctctcatg tctactgtg
94351  cctttccctc actttcctit tctgtcaaac tcatgaacca tggggctcag
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94451  ggt333333tgc atgggaact agatgtctta scatctctcc ccttcagtg
94501  atgtctcatt gcttcaaat gcagaggttc ttaatccttg ttgatgttct
94551  ttgttaaacc attgagaat tgatggaaat tatgggtct tctccagaa
94601  agtatctcaa tgcacgagaa atcaattact ttgctacaa ttttaattga
94651  tncacagagt cctgaagcc agttcaaac ttttgttta caaagcaaac
94701  tctgtttaat caagtgaac taattttcag tgaattagag ggaattttt
94751  acatttaagt ggettaccga atggagagt ttgtagttag aacagacta
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94851  gaacttctga tgggaagat catttatcca gtcatgtatg cattcaacaa
94901  ttactcattg agcaactat atgtgcagg catagtctta ggcactgaga
94951  atgaagcagt tgggtt3333 aaaaaaaa agttgaatc tctggcttg
95001  ggaagtatgt attcaagga cgttgggata gacaataaa ataaataat
95051  ataggatatg ttagatggca ttaagtctta aggagaanaa taaataaag
95101  aagg333333tga gggagcates gaggetataa tttcaatag gtggccaggg
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95251  catagtggga tcttttgatt ccaat333333a gga333333aga gaggtctaa
95301  aaacagctgt gcagggcaga gagaatggg agggaggaca ctcaacctg
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95401  cctcttacct acadtbtaga ggtcttagca attctcttc aaagtcttat
95451  tttataaca gaaggatgt tagaagcaag tactttctt gatttctctc
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95551  aggggaatgag tca333333attc tatgaggtta tggggaatta tcaagctcca
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95751  cctgtgtgct tagacctca ttgtctaaa tgagttacta caaatgccac
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95851  tactccaagg aatttttgat ttatacgt sc333333333 atctgctca
95901  gcaagtgar gagateaat tcaataatga atccaggtt agaaacc333
95951  gtctgccaga tgcacagaga acagttatgt tgtactaga aacattctaa
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96801  atatatatat atatatatat atatatatat atatatatat atatatatat
96851  tatacatata cacatcctc cattatatgt atagtatat atacctatc
96901  atataatct ttttttttgg caagatgaa ccaactctct gtgcattct
96951  cagatctcca gccacgtgag tctgaggto tactraagt actca333333ag

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Exon 9

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97151 tgatctgtgt taatgagtag caggaagtgc cragatttga gagcaaaagc
97201 ctgaaaaaca gaggccaggtg aaaccagta ttaatgatgt gaagagtggc
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97301 acacattttt tttctctaca ttgtcactgt gtttccataa agaatgaag
97351 gcctccacot cccagaggtt ggtccctaaq tggaggccag tctgggccc
97401 gggattgttc ttttagctta gtactattat aataattata ttaataataa
97451 tatattttat caatactgaa gccagttttg aaagtccact tgggtgtatta
97501 aggtctctga ttatccattt ttgcactatg tttatatgta aagataadca
97551 aatgaaaaaa taatgcctc cgtctatgat gggagatagg cacgatagga
97601 tatacaacat caggccacatc ttctgcttag gtaacctacc caagggatgg
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Exon 10

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FIGURE 1AM

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Exon 11

Exon 12

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Exon 13

Exon 14

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KCN6 α cDNA

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101	CCTGAGGAGAGCCGCGGGGCAAGCAGGGGGCCCGGATGAGCCTGCTGG	150
1	M S L L G	5
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22	Y R R V Q N Y L Y W V L E R P R G	30
251	CTGGGCGTTCACTACCAAGCTTTCCTTTCTCTTCTGCTTTGGTTGCT	300
39	W A F I Y H A F V F L L V F E C L	55
301	TGATTTTCCAGTGTTTCTACCATCCCTGAGCAGACAAATTGGGCTCA	350
56	I L S V F S T I P E H T K L A S	71
351	AGTTGCCTCTTGAATCTGGAGTTCTGTGATGATTGCTGCTCTTTGGTTTGG	400
72	S C L L I L E F V M I V V F G L E	88
401	GTTCATCATTCGAATCTGGTCTGCGGSETTGCTGTTGTGATATAGAGGAT	450
89	F I I R I W S A E C C C R Y R G W	105
451	GGCAAGCAAGACTGAGGTTTGCCTGAAAGCCCTTCTGTGTTATAGATACC	500
106	Q G R L R F A R K P F C V I D T	121
501	ATGTGCTCTATCGCTTCAATAGCAGTTGTTTCTGCAAAAACCTCAGGGTAA	550
122	I V L I A S I A V V S A K T Q G N	138
551	TATTTTGGCAGCTCTGCACTCAGAAGTCTCCGTTTCTACAGATCCTCC	600
139	I F A T S A L R S L R F L Q I L R	155
601	GCATGCTGCGCATGGACCGAAGGGGAGGCACTTGGAAATCACTGGGTTCA	650
156	M V R M D R R G G T W K L L G S	171
651	GTEGTTTATGCTCAGCAAGGAATTAATCACAGCTTGGTACATAGGATT	700
172	V V Y A H S K E L I T A W Y I G F	188
701	TTGCGTTCTTATTTCTCTCTTCTCTATCTGCTGCAAAAGGATG	750
189	L V L I F S S F L V Y L V E K D A	205
751	CCAATAAAGAGTTTCTACATATGCAGATGCTCTCTGGTGGGGCACAATT	800

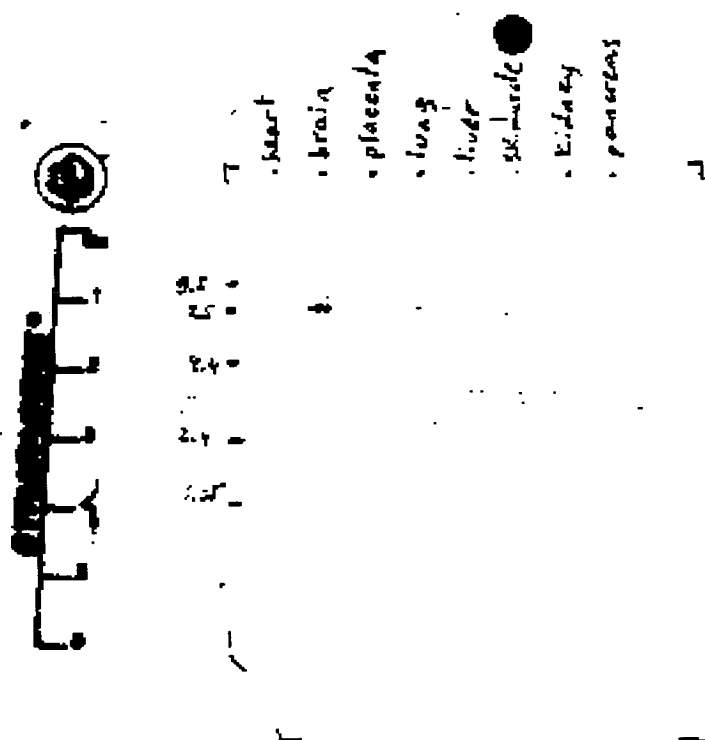
206	N K E F S T Y A D A L N W G T Y	221
601	ACATTGACAACTATTGGCTATGGAGACAAACTCCCCCTAACTTGGCTGGG	950
222	T L T T I G Y G D K T P L T W L G	238
851	AAGATTGCTTTCTGCAGGCTTTGCACTCCTTGGCATTTCCTTTCTTTGCAC	900
239	R L L S A G F A L L G I S F F A L	255
901	TTCTTGGGGGCACTTCTTGGCTCAGGTTTTCGATTAAAGTACAAGAACAA	950
256	P A G I L G S G F A L K V Q E Q	271
951	CACCGCTAGAAACACTTTGAGAAAGGAAGGAACCCAGCTGCTAACCTCAT	1000
272	H R Q K H F E K R R N P A A N L I	288
1001	TCAGTGTGTTTGGCGTAGTTACGCAGCTGATGAGAAATCTGTTTCCATTG	1050
289	Q C V W R S Y A A D E K S V S I A	305
1051	CAACCTGGAGGCTACACTTGGAGGCTTGCACACCTGCAAGCCCTACCAAG	1100
306	T N K P H L K A L H T C S P T K	321
1101	AAAGAACAGGGGAAGCATCAAGCACTCAGAGGCTAAGTTTTAAGGAGGG	1150
322	K E Q G E A S S S Q K L S P K E R	338
1151	AGTGGGCTAGGCTAGCCCCAGGGGCCAGAGTATTAAAGAGCGGACAAAGCCT	1200
339	V R M A S P R G Q S I K S R Q A S	355
1201	CAGTAGGTGACAGGAGGTCGCCAAGCACCGACATCACAGCCGAGGGCAGT	1250
356	V G D R R S P S T D I T A E G S	371
1251	CCCACCAAAGTGCAGAGAGGCTGGAGCTTCAAGGACTGAACCCGCTTCGG	1300
372	P T K V Q K S W S F N D R T R F R	388
1301	GCCCTGGGTGCGCCTCAAAAGTTCTCAGCCAAAACCACTGATAGATGCTG	1350
389	P S L R L K S S Q P K P V I D A D	405
1351	ACACAGCCCTTGGCACTGATGATGATATATGATGAAGAGGATGCCAGTGT	1400
406	T A L G T D D V Y D E K G C Q C	421
1401	GATGTATCACTGGAAGACCTCACCCCACTTAAACTGTCAATTCGAGC	1450
422	D V S V E D L T P P L K T V I R A	438
1451	TATCAGAATTATGAAATTTCTATGTTGCAAAACGGAAAGTTTAAAGGAACAT	1500
439	I R I M K F H V A K R K F K E T L	455
1501	TACGTCCATATGATGTAAAAGATGTGATTTGAACATATCTGCTGGTCAAT	1550
456	R P Y D V K D V I E Q Y S A G H	471

1551	CTGGACATGTTGTGTAGAATTAAAGCCTTCAAACACGTTGATCAAAT	1600
472	L D M L C R I K S L Q T R V D Q I	468
1601	TCTTGGAAAGGCGCAATCAGATCAGATAGAGAGCGGAGAGAAATTA	1650
489	L G K G Q I T S D K K S R E K I T	505
1651	CAGCAGACATGAGACCCACAGACGATCTCAGTATGCTCGGTCGGGTGGTC	1700
506	A E H E T T D D L S M L G R V V	521
1701	AAGGTTGAAAAACAGGTACAGTCCATAGAATCEAAGCTGGACTGCCTACT	1750
522	K V E K Q V Q S I E S K L D C L L	536
1751	AGACATCTATCAACAGGTCCTTCGGAAGGCTCTGCCTCAGCCCTCGCTT	1800
539	D I Y Q Q V L R K G S A S A L A L	555
1801	TGGCTTCATTCCAGATCCGACCTTTTGAATGTGAACAGACATCTGACTAT	1850
556	A S F Q I P P F E C E Q T S D Y	571
1851	CAAAGCCCTGTGGATAGCAAGATCTTTCGGGTTCCGCACAAAACAGTGG	1900
572	Q S P V D S K D L S G S A Q N S G	588
1901	CTGTTTATCCAGATCAACTAGTGCCAAACATCTCGAGAGGCTTGCAGTTCA	1950
589	C L S R S T S A N I S R G L Q F I	605
1951	TTCTGACGCCAATGAGTTCAGTCCGACACTTTCTACGCGCTTAGCCCT	2000
606	L T P N E F S A Q T F Y A L S P	621
2001	ACTATGCACAGTCAAGCAACACAGGTGCCAATTAGTCAGAGCGATGSETC	2050
622	T M H S Q A T Q V P I S Q S D G S	638
2051	AGCAGTGGCCAGCCACCAACCATTTGCCAAACCAATAAATACGGCACCCA	2100
639	A V A A T N T I A N Q I N T A P K	655
2101	AGCCAGCAGCCCAACAACCTTTACAGATCCACCTCCTCTCCAGCCATC	2150
656	P A A P T T L Q I P P P L P A I	671
2151	AAGCATCTGCCAGGCCAGAACTCTGCAACCCCAACCCCTGCAGGCTTACA	2200
672	K H L P R P E T L H P N P A G L Q	688
2201	GGAAAGCCTTTCTGACGTCAACACCTGCTTGTGCTTCCAAGGAAAATG	2250
689	E S I S D V T T C L V A S K E N V	705
2251	TTGAGGTTGCACAGTCAANTCTCACCAAGGACCGTTCTATGAGGAAGG	2300
706	Q V A Q S N L T K D R S M R K S	721
2301	TTTGACATGGGAGGAGAACTCTGTGTCTGTCTGCCATGGTSCCGAA	2350

722	F D M G G E T L L S V C P M V P K	738
2351	GGACTTGGGSCAAATCTTTGTGTGCAAAACCTGATCAGGTGACCGAGG	2400
739	D L G K S L S V Q N L I R S T E E	755
2401	AAGTGAATATACAACTTTCAGGGAGTGAATCAAGTGCCTCCAGAGGCAGC	2450
756	L N I Q L S G S E S S A S R E S	771
2451	CAAGATTTTTACCCCAATGGAGGGAATCCAAATTTTATTAAGTATGA	2500
772	Q D F Y P K W R E S K L F I T D E	788
2501	AGAGGTGGGTCCCGAAGAGACAGAGACAGACACTTTTGATGCCGCGCCGC	2550
789	E V G P E E T E T D T F D A A P Q	805
2551	AGCCTGCCAGGGGAAGCTGCCCTTTGCATCAGACTCTCTAAGGACTCGAAGG	2600
806	P A R E A A F A S D S L R T G R	821
2601	TCAGGATCATCTCAGAGCATTGTAAAGGCAGGAGAAAGTACAGATGCCCT	2650
822	S R S S Q S I C K A G E S T D A L	838
2651	CAGCTTGCCCTCATGTCAAAGTGAATAAGTCTTTCATTTTCTTCCAGGC	2700
839	S L P H V K L K	846
2701	ATAGCAATTCTTTAGCCATACATATCATTTGCATGAAGTATTTGGAAGCC	2750
2751	CTTCTAAAGAGTTGAAATTCGAAGATCGGGAAGAACATGAAGGCAGTT	2800
2801	TATAAGCCCGTTACCTTTTAATTCGATGAAGATGCATGTTAGGGATGGC	2850
2851	TAAATTCCTAGGTGCATCGACATTACCCCACTCATTAGTAATGTACCT	2900
2901	TGAGTTAAAGGCCTGAGAAACCAACACAGCTAATGCTATGGGCTGTAT	2950
2951	GAAATATGTCAAGTTTAGGTCATTAGAGATTGACACTGTATTTTGAA	3000
3001	TTATGGGAGTAAACACCTTCAAAATTTCAAGGCATTTCTGCTTTGTACTAA	3050
3051	ATACAAACTACATTTTCAAGATTAGGCCATAATGTATATTTAAACACAAT	3100
3101	GGCTATCAACAGCTGCTAATAAGGTATCAACTAAAGCAGAAATGGGGAAT	3150
3151	AATAGAAATGGGTGCTTATTTCAAGATATATTTGCCAACCCTTCTCTATT	3200
3201	CAGTCATTTTATTATTAATGTAATTTGAATGTCAACTTGCTGTGCTTTTGG	3250

3251	TGATTTAGCGCTGTGGCAAGCAATTTTGCACATCATTTTCATGTTGTTCT	3300
3301	TTATGACAAGAATGTTCTTCAATTAGAAAATGTGCAATTAATGAAATTC	3350
3351	GGGCCAGTGAGGCAATAGACTATCTGACATATTTGACTTTATGAARAC	3400
3401	TATTGCCCTGATGGCAGAAATCAACTTCATAAGTGGTCRACTTCTACACAAG	3450
3451	CGTATGAAATACTGGTCAGTAGAACAGCCATTGTGATGGACTGTTTCT	3500
3501	CTGCAATGGCGCCAACCCGAGGCTTGCCAATACTGCCCTATGTAAAGGGCA	3550
3551	AGTGTGAGAGCTATTCTCATTTCGCTGACATACAGGTAGGACTATGEGG	3600
3601	GATGGGACATTTGAGTGGGACTGAGATAGGGAAGGCTTGAAAGAAACCA	3650
3651	GAACACCCGCCAGGAAGTTGGCAAGTAAAAGAAAATGACTTCCCGCTCA	3700
3701	AAGGGCAATGAGAGGGAG	

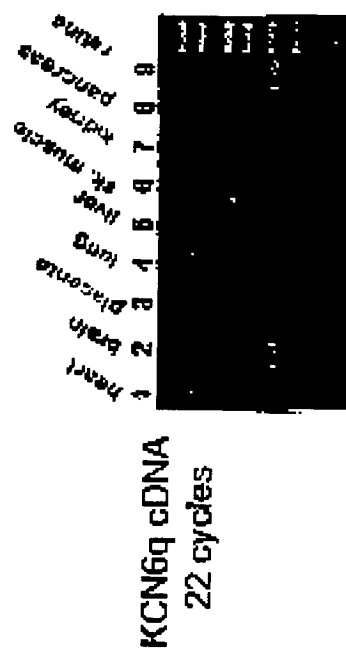
FIGURE 3A



probe: DL/ER per fragment.

FIGURE 3B

RT-PCR analysis of the KCN6q gene expression in human tissues



[illegible]

FIGURE 4B

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus ma k rz paaq ss dg qgaa pag ai ag er vol ppaq g alv

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus acpr a lqapls gagr garrtp gqllakte s kaaakayrryrl

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus yvllerpqgacfihaavlliv clilavlatikayakiasqcllii vmiyvqlz

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus yivriwsagccoxrrow orlrfakrfpovidilvliabavvaagccqgvfiatsalral

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus rflqilrmvrmrroctwklldvvyahskellitawytgflvllifssilvttlaed v

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus daqq andofstyalalwglilfittieygdxtptwlgzila flligisffalpac

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus ilsgcfalavqeqhraqhfekrtzpaanliqaawrzatd per dl atnkyys val

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus p fklp l qtarnggllrplevrrapvpgaprrypvatostpg afk

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus p epssqkiskidrvrmaspqrqvp gkl tap tarrspatd fctapky kaw

KCN6q_...
KCNQ4_...
KCNQ2_...
KCNQ3_...
KCNQ1_...
consensus sindtrart rikgapr vqs ada pggavadek qodfavadtalk vira

[illegible]

